VOLUME 35 MARCH 1957 NUMBER 2

Canadian Journal of Botany

Editor: D. L. BAILEY

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF BOTANY

(Formerly Section C, Canadian Journal of Research)

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Canadian Journal of Botany

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 35 MARCH 1957 NUMBER 2

PHOTOPERIODIC RESPONSES IN SEEDLINGS OF NORTHERN TREE SPECIES[1]

O. VAARTAJA[2]

Abstract

Seedlings of 10 tree species were tested under two widely different photoperiodic conditions in environment otherwise near optimum. The amount of light given to the plants was the same under both conditions. Under the conditions simulating long photoperiods, the seedlings of all the species grew well and continued their growth during the 8-month experimental period. Under the short photoperiod, the following species remained nearly or fully dormant for several months beginning soon after germination: *Larix laricina* (Du Roi) K. Koch, *Ulmus americana* L., and two provenances of *Picea glauca* (Moench) Voss; the following species either remained nearly dormant or grew significantly slower than under the long day conditions: *Betula lutea* Michx. f., *B. verrucosa* Ehr., and *Pinus banksiana* Lamb.; *P. resinosa* Ait. was considered sensitive to photoperiods but its responses in appearance and growth were small; *Caragana arborescens* Lam., *Thuja plicata* Donn, and *Acer negundo* L. showed no significant response.

Introduction

The importance of photoperiods in the growth of many tree species has been well established. Literature on this fundamentally interesting phenomenon has been recently reviewed by Wareing (6, 7, 8), Olmsted (2), Pauley and Perry (3), and Vaartaja (4, 5). The growth of many tree seedlings is promoted by long photoperiods and very short photoperiods tend to induce abnormally early dormancy.

The great importance of these photoperiodic phenomena in tree breeding is obvious (3, 5). They should also be considered in any research involving the growth of trees, especially in growth chambers and greenhouses. The short natural photoperiods prevailing in northern countries in winter may inhibit the normal growth of tree seedlings unless extended artificially. Even a light of short duration and of relatively low intensity in the middle of the dark period may ensure normal or better than normal growth (7, 8, 9). This is because the decisive factor is the duration of the dark period and not the cumulative effect of repeated dark periods. This has been found also in the development of many herbaceous plants (1).

Very little is known of the photoperiodic responses of Canadian tree species. Therefore the following observations on the responses of nine Canadian species to two photoperiods is of particular interest. They provide badly

[1]Manuscript received November 14, 1956.
Contribution No. 327, Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada.

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[The previous number of Can. J. Botany (35, 1–131) was issued February 14, 1957.]

needed, though still fragmentary, information which will serve until more comprehensive investigations can be made. The information obtained was actually needed for certain research projects to be conducted in a greenhouse. A Finnish species known to react sensitively to photoperiods (4) served as a check.

Methods

Seed of each of the following species was sown in four 8-inch pots in a mixture of peat, sand, and fertilized loam: (1) Manitoba maple (*Acer negundo*) from Indian Head, Sask. (cultivated, the original source not known); (2) caragana (*Caragana arborescens*) from Sutherland, Sask. (cultivated, the original source not known); (3) yellow birch (*Betula lutea*) from North Bay, Ont.; (4) birch (*B. verrucosa*) from Helsinki, Finland; (5) white elm (*Ulmus americana*) from Indian Head, Sask. (cultivated, original source not known); (6) western red cedar (*Thuja plicata*) from Nelson, B.C.; (7) red pine (*Pinus resinosa*), mixed seed from western Ontario; (8) jack pine (*P. banksiana*), mixed seed from eastern Ontario; (9) tamarack (*Larix laricina*) from Canwood, Sask.; (10*A*) white spruce (*Picea glauca*) from Candle Lake, Sask.; (10*B*) white spruce from Aleza Lake, B.C. Two pots of each species were put in one compartment of a greenhouse and two in another, all the pots in a random order in each compartment.

In one compartment long day conditions (= L treatment) were simulated. During the first experimental month, when most of the germination and little growth took place, the natural short day (of late August and early September in Saskatoon, Sask.) was supplemented by continuous fluorescent light[3] of 100 ft-c. This was sufficient for the photoperiodic responses (1, 5, 7) but added only a negligible amount to the natural light, which commonly reached the intensity of 5000 ft-c. Later, in addition to the gradually declining (seasonal) natural light, fluorescent light[3] of 500 ft-c. was provided for 13 hours during the day and for 1 hour at midnight. This interrupted and divided the dark period, otherwise 11 hours long, into two 5-hour periods.

In the other compartment short day conditions (= S treatment) were provided. The natural photoperiod of approximately 14 hours was not modified during the first experimental month. Later the natural daylight was supplemented with fluorescent light[3] of 500 ft-c. for 14 hours during the day.

The amount and total duration of the light were practically the same under L and S but the photoperiodic conditions were quite different. L treatment (5-hour night) corresponded to photoperiodic conditions in midsummer in latitude 60° or at the southern border of the Yukon and Northwest Territories or in South Finland. S treatment (10-hour night) corresponded to those in midsummer in latitude 30° or in the southern part of the United States. Most of the experimental seed originated between latitudes 47° and 54° (45° for jack pine and 60° for the Finnish birch). Between latitudes 47° and 54°, the extreme long day conditions corresponding to L never occur

[3]From General Electric cool white type fluorescent tubes.

naturally; conditions corresponding to S occur only before and after the frostless warm season. These photoperiodic conditions were chosen, assuming that they would clearly show whether the experimental seedlings respond photoperiodically. A series of various photoperiods would be necessary for a more thorough study; this was not possible in this study.

Both greenhouse compartments were maintained at approximately the same daily temperature cycle. This varied usually between 15° and 30° C. Soil in pots was kept moist continuously through frequent sprinkling with tap water. Thus, temperature and moisture were never far from the optimum.

The growth and morphological characteristics of the seedlings were recorded periodically during 8 months. The growth data for 7 months after sowing were analyzed statistically. These data were based on eight coniferous and six hardwood individuals in each of the four pots of each species.

Results

Some mortality of seedlings caused by damping-off occurred soon after germination. One purpose of the study was actually to investigate the effects of photoperiods on mortality. However, the mortality varied irregularly and, in the soil used, inoculation with a virulent pathogen may be necessary for significant results in mortality.

One month after germination the seedling stands were thinned to provide uncrowded growing space for the remaining seedlings. The fast growing hardwood species were thinned three times. The mortality and thinning may have exerted selection in the populations. Therefore the results should not be assumed to reflect the responses of any total populations, but rather the potentials of these to adapt to two extreme conditions such as L and S. However, no marked individual variation was noticed in any of the conifer species although they germinated in large numbers (200–400 within a treatment) and were ocularly inspected before thinnings. There was noticeable variation in growth of hardwood species in L but this might have been due to the crowding as well as to the genetic variation.

The two photoperiodic conditions, on the other hand, caused large differences in the form and development of spruce and tamarack soon after germination. Under S these became dormant or nearly dormant after the cotyledons and a few small juvenile needles had developed. The same happened with elm after one to three small leaves had developed (Fig. 1, S_1). Later a few more juvenile needles developed in spruce and tamarack. Because the height growth was negligible, the needles gave a rosette-like appearance to the seedlings (Fig. 1, S_3 and S_4). After 5 to 8 months terminal buds of a few spruce and elm seedlings finally burst and considerable height growth took place (Fig. 1, S_4 and S_1). Peculiarly, in some spruce individuals a new leader developed from a lateral bud while the terminal bud remained nearly dormant.

Similar but not quite so consistent responses in the stages of growth were observed with the pines and birches under S. Some branches of jack pine developed more vigorously than the terminal leader (Fig. 1, S_2). Most birch individuals appeared quite dormant at first but later grew slowly.

Under L all species grew continuously or had only a brief resting period with a temporary terminal bud between vigorous growth periods. Fig. 1(L_1-L_4) illustrates the growth of some species.

Table I summarizes the responses in development and demonstrates the responses in growth as recorded 7 months after sowing. To make it possible to analyze the heterogeneous data on heights, these were divided into four uniform parts as shown in the table, and statistical analysis was made separately for each part. The growth differences between L and S illustrate the large variation between species in the degree of photoperiodic sensitivity. The differences in the responses of red pine were not so clear 7 months after sowing as they were earlier. This sample was considered to be sensitive to photoperiods although the growth data of the table do not show significant differences.

Three of the 10 species did not show any marked photoperiodic responses to the conditions used. Yet even they might respond somewhat if tested with still shorter photoperiods. Actually some preliminary experiments with very short photoperiods (natural winter day-length in Saskatoon) suggested slight photoperiodic responses in caragana.

Discussion

Dependence of the onset of full or nearly full dormancy on short photoperiods was demonstrated with tamarack and two samples of white spruce (Table I). The seed sources of these trees suggest that they represented genetic pools originating entirely or mostly north of the prairies. As discussed

TABLE I

INFLUENCE OF TWO PHOTOPERIODIC CONDITIONS (L AND S) ON GROWTH AND DEVELOPMENT OF TREE SEEDLINGS IN 7 MONTHS IN GREENHOUSE; L CORRESPONDS WITH LONG DAY CONDITIONS AND S WITH SHORT DAY CONDITIONS

Species	L[a] height[c] in mm.	S[b] height, per cent of L	Terminal bud dormant[d] under S	No. buds greater in L than S	No. leaves greater in L than S
Acer negundo	248	107	−	−	−
Caragana arborescens	167	98	—	—	—
Betula lutea	80	32*	−	−	+
Betula verrucosa	169	53*	−	+	+
Ulmus americana	154	38*	+	−	+
Thuja plicata	24	82	−	−	−
Pinus resinosa	17	55	−	−	−
Pinus banksiana	26	39*	−	−	+
Larix laricina	66	1.5**	+	+	+
Picea glauca A	30	4.0**	+	+	+
B	57	6.3**	+	+	+

[a]L = light 13 hours during day and 1 hour at midnight.
[b]S = light 14 hours during day.
[c]Height of conifers measured from cotyledons to top of main stem: Height of hardwoods from ground to top of main stem.
[d]At the time of inspection, 7 months from sowing.
**P <0.01. *P <0.05.

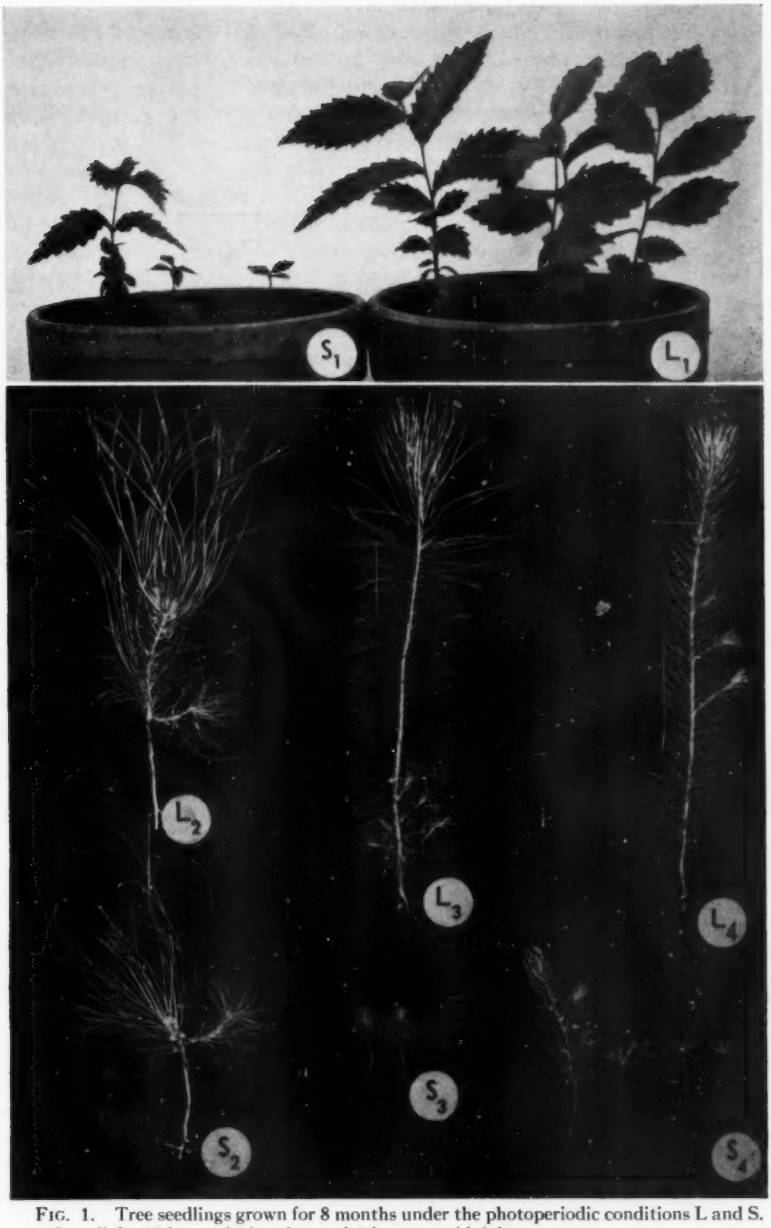

FIG. 1. Tree seedlings grown for 8 months under the photoperiodic conditions L and S.
L = light 13 hours during day and 1 hour at midnight.
S = light 14 hours during day.
1 = *U. americana;* 2 = *P. banksiana;* 3 = *L. laricina;* 4 = *P. glauca.*

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earlier (5), in far northern latitudes under drastic seasonal extremes of photoclimate, the photoperiods can control the onset of dormancy with special advantage and efficiency. This indirect mechanism of trees to adapt to the yearly weather cycle favors the selection of ecotypes fully adapted to the general climate of the site. Without this mechanism an incidental warm period would favor ecotypes that are fast growing but not hardy. The studies so far made suggest that this mechanism has been developed, during phylogeny, by many truly northern tree species. Like *Larix laricina* and *Picea glauca* from Canada, *Larix sibirica* Leb. (unpublished details from (5)) and *Picea abies* (L.) Karst. (4, 5) from Finland show strict dependence upon photoperiods. This was true also with a far northern ecotype of *Alnus incana* (L.) Moench (5) and, in certain tests under very short photoperiods, with *Betula pubescens* Ehr. and *B. verrucosa* Ehr. (4). *Populus tremula* L., *Alnus incana* Gaertn., and *Pinus sylvestris* L. from Finland also react very sensitively to short photoperiods (4, 5).

Combining, for a long time, the optimum temperatures and short photoperiods is an unnatural condition for northern trees. This may explain the unnatural growth pattern of a few individuals of spruce and jack pine seedlings kept under these conditions for several months (Fig. 1). The optimum growth environment may also have caused individuals of elm and spruce finally to break their dormancy in a normal pattern despite the obvious earlier effects of short photoperiods. This environment with factors working in opposite directions may also explain the half-dormant stage and poor growth of most of the experimental seedlings under S treatment (Table I). A somewhat different combination of these factors may cause full dormancy in some of these species as has been shown (4) with *B. verrucosa*. On the other hand, the possibility remains that with some of the species tested, the onset of full dormancy requires action by two mechanisms, one based on short photoperiods and another on low temperatures. According to Olmsted (2) this applies to sugar maple (*Acer saccharum* Marsh.), for instance.

Species with wide climatic ranges likely contain different photoperiodic ecotypes (5). Therefore, the photoperiodic responses of tamaracks taken from the southern extremities of its continuous range in Indiana, Pennsylvania, or New Jersey, and especially from the isolated occurrences still farther south, might be quite different from those demonstrated in Table I. This deserves special attention in future research with this species or other species having very wide climatic ranges or separate ranges.

As expected, all the trees tested grew well under L treatment although the photoperiodic conditions corresponded to a longer day than the longest natural day at their seed source. This has also been found in numerous other studies (5) even with species from such short day conditions as those in North Carolina (9). In Wareing's (7) test, the growth of Scots pine (*P. sylvestris*) was less under continuous light than under an optimum photoperiod, but the difference was very small.

The good growth of tree seedlings under long photoperiods can be utilized in greenhouses. This is useful in many kinds of research and possibly also in practice when valuable tree seedlings are grown. The addition of the necessary midnight light period does not add much to the costs otherwise encountered in greenhouse practice.

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THE DEVELOPMENT OF THE BLUEBERRY SEED[1]

HUGH P. BELL

Abstract

Seed development was followed from fertilization to maturity. Pollen tubes required about 4 days to grow from stigma to ovule. In some plants, particularly bagged ones, nucellar cells remained alive and contents of the embryo sac degenerated. Many ovules did not develop. Seeds were counted and sorted in a random representative collection of 1075 berries. The average number of seeds per berry was 64.2. Of these 49.9 (or 77.7%) were imperfect. More complete pollination increased the percentage of normally developing ovules. Development of perfect seeds followed a familiar pattern. Unfamiliar features were noted as follows: 1. Degeneration of cells at both micropylar and chalazal ends resulted in a homogeneous plasma. This plasma formed strands across haustoria and almost completely surrounded the zygote. 2. Micropylar endosperm cells formed a dense plug. Developing embryos may have had difficulty in penetrating this plug. 3. Many embryos had died at some stage of development. 4. A conspicuous integumentary tapetum was present until the endosperm was about half its final size.

Embryo development was the "soland" type. Mature seeds were "axile linear". Imperfect seeds were chiefly of two types: (*a*) medium sized and solid with middle integumentary layers lignified, or (*b*) small and collapsed with all tissues inside seed coat disintegrated. No imperfect seed had an embryo.

Introduction

The work reported below is a continuation of that published in 1955 (3) which covered the early development of the flower in the lowbush blueberry. As in the previous article the species described is *Vaccinium angustifolium* Ait. var. *laevifolium* House.

Materials and Methods

The killing fluid used was Nawaschin's, and the stain iron alum haematoxylin. At times during the investigation the material presented certain technical difficulties. The techniques employed for overcoming these difficulties will be outlined when the particular structures are being described.

Rate of Pollen Tube Growth

The study of the rate of pollen tube growth was started in 1954 and continued in 1955. In each year about 50 unopened flowers were selected. These were bagged in the usual way with waxed paper. A certain number each year were pollinated as soon as the flower opened, then rebagged and the time of pollination noted. None of the unpollinated bagged flowers set fruit. All of the artificially pollinated ones did. These were collected at regular intervals after pollination, killed immediately, sectioned, and examined. The desired information was provided by serial transverse sections. It was found that it took between 3 and 4 days for the pollen tube to grow from the stigma to the ovules.

[1]Manuscript received November 5, 1956.
Contribution from the Nova Scotia Research Foundation, Halifax, Nova Scotia.

The Effect of Bagging on the Development of the Ovule

In the material from bagged plants which were pollinated and had set fruit a very unusual structure or form of development was observed. This involved a complete reversal of the usual fate of embryo sac contents and nucellus, that is, in ovaries in which there were normal embryo sacs with mature female gametophytes and the nucellus completely degenerated, there were always a number of ovules in which the nucellus had remained alive and active but the embryo sac contents had degenerated (Fig. 1). This fact suggested that the bagging with wax paper had a detrimental effect on the embryo sac contents, so in 1956 a certain number of flower buds were bagged but not pollinated. Ovaries from these buds were collected shortly after the flowers opened. The degeneration of embryo sac contents and continued activity of the nucellus was again found in a number of cases. This reversal of the activity of the nucellus and embryo sac contents occurs in nature, for this structure was found in buds grown in the open where they had not been subjected to any artificial interference, but its more frequent occurrence in the bagged plants suggests that bagging with waxed paper is not a satisfactory method for controlling pollination with the blueberry.

Frequent Non-development of Ovules

One of the difficulties encountered early in this work was to find a sufficient number of normally developing ovules. In most ovaries sectioned there would be very few ovules developed beyond the fertilization stage, and many of the ovules would not even be fertilized. This non-development of so many ovules suggested that there must be a low average of viable seeds per ripe berry. To check this, counts were made from a random sample of 1075 berries. To check the suitability of the sample the diameters of these berries were measured with micrometer calipers and the berries were divided into size groups for every half millimeter difference in diameter measurement. The number in each size group was counted and these figures were plotted. They gave an almost perfect probability curve so the collection was regarded as a representative one. Then the seeds were extracted and sorted into groups of perfect and imperfect seeds, and these were counted. There was one fact which was obvious, namely, that the larger berries had a much greater proportion of perfect seeds. This finding was in agreement with evidence that had already been obtained at the Experimental Station in Maine. This evidence had been kindly sent to us by Dr. M. T. Hilborn (9) of that station. But in the sample used for the present investigation there were very few berries in the large and small groups; hence the figures are not significant for these groups, so these figures are not given. The only significant figures are those for the collection as a whole. These are as follows:

Total number of seeds all types	69,092
Total number of perfect seeds	14,395
Total number of imperfect seeds	54,747
Average number of seeds per berry	64.2
Average number of perfect seeds per berry	13.3
Average number of imperfect seeds per berry	49.9

That is, on the average, 77.7% of the ovules do not develop. These figures explain why it was so difficult to obtain an adequate number of normally developing ovules in the average ovary.

The real problem to be solved was to obtain ovaries with a larger number of normally developing ovules, otherwise it would have been necessary to section an enormous number. The most likely solution seemed to be to ensure more adequate pollination. This supposition was supported by the position of the large perfect seeds in each berry. These were always clustered around the top of the central axis with the imperfect seeds occupying the lower and basal portion of the loculi, suggesting that there had been sufficient number of pollen tubes to fertilize, not all the ovules, but only the ones first encountered as the pollen tubes entered the loculi. Better pollination was accomplished, first by collecting in a field adequately supplied with beehives, and then by covering with fresh viable pollen the stigmas of flowers which had just opened. The most thoroughly pollinated plants provided the best material for ovule development and this material was adequate.

Description of Development

I. General

The descriptions given below start with fertilization and continue to the mature seed, that is, they are a continuation of a previous article (3) which described the stages to and including the mature embryo sac. Other articles have been published which deal in a similar manner with embryo and seed development for a number of the Ericaceae and in 1951 Crété (7) summarized the information then available on the development of haustoria, but in none of these is the story continuous and complete for any one species. These other articles, however, include many excellent illustrations. Where these fit into the following outline they will be cited to supplement the illustrations in the present article. At any stage in this development and especially in the mid-stages a number of changes take place concurrently. For clarity these will be described under different headings but to have a clear picture of the whole developmental process it must be remembered that many of the structures described separately are developing at the same time. To make these separate descriptions clear and then to correlate them, a certain amount of repetition is unavoidable.

II. Early Stages

(*a*) *Fertilization*

The pollen tube enters by the micropyle (Fig. 2) usually destroying one of the synergids. The tip of pollen tube when inside the embryo sac takes the stain so deeply that it is black and opaque. At the same time, in the vicinity of the egg, numerous minute black bodies appear. Thus the male cell could not be identified before the beginning of fertilization. Then one of the male cells may be seen after it has entered the egg, and the other male cell, having become associated with the polar nuclei, may be identified lying either beside them or between them (Fig. 2). These three nuclei gradually merge (Fig. 3)

forming the triple fusion nucleus (Fig. 4) but the egg and the male cell do not unite until some stage after the primary endosperm nucleus has started endosperm formation. For instance, Fig. 5 illustrates a primary endosperm nucleus starting to divide but the male cell is still distinct in the cytoplasm of the egg.

(*b*) *Initiation of Micropyle Haustorium*

Concurrently with the entrance of the pollen tube into the embryo sac there is an enlargement of the region between the micropyle and the constriction formed at the anterior end of the endothelium (Fig. 2). This region now contains the zygote (or egg and male nucleus), several minute black bodies, the end of the pollen tube, a degenerated synergid, and a second synergid which may be alive or also dead and disintegrating. The space in which these bodies are located is by this time a distinct cavity and it is the start of the micropyle haustorium.

(*c*) *Initiation of Chalazal Haustorium*

The initiation of a haustorium at the chalazal end of the embryo sac is not such a clear-cut picture as it is at the micropyle end. At the chalazal end it may start merely by the death of the antipodals and the gradual migration of the embryo sac protoplasm into the region which they occupied (Figs. 2, 7, and 8) or the antipodals may by disintegrating form a more or less continuous granular mass around the tip of the invading protoplasm. If this takes place the ovule cells at the chalazal end disintegrate and become crushed (Fig. 9). In either case the chalazal haustorium has been initiated.

(*d*) *Endosperm*

Immediately after triple fusion is completed the primary endosperm nucleus divides (Fig. 5) forming two free nuclei (Fig. 6). Each of these divides again, the micropylar one slightly in advance of the other (Fig. 7). This division is also nuclear (Fig. 8) and is thus in agreement with Peltrisot's (15) description, but Samuelsson (16) thinks that Peltrisot's interpretation may not be correct, for in most Ericaceae walls are laid down immediately between the first formed endosperm nuclei. Maheshwari (12) is in agreement with Samuelsson. Recently Creech (6) found a row of four free nuclei in *Rhododendron*. With the additional evidence brought forward in the present paper it would appear that in certain Ericaceae there is a very brief period when the endosperm is nuclear.

III. Mid-stages

(*a*) *Endosperm*

The formation of endosperm tissue starts immediately after the four free nuclei stage. Then walls are laid down giving a four-celled endosperm (Fig. 9) and this is followed by a longitudinal division in each of these cells giving an eight-celled endosperm in which the four central cells are differentiated from the two at each end (Fig. 10). These four central cells continue to divide until there are about 18 cells when the cells at the micropyle end multiply more rapidly, do not enlarge, and become richly protoplasmic. These form a

dense plug at the entrance into the micropyle haustorium (Figs. 19, 21, 22, 23, and 28). This plug was observed and described by both Artopoeus (1) and Peltrisot (15) and Peltrisot in his Figure 5 illustrated it for *Vaccinium uliginosum*. The cells in the main or central portion of the endosperm at this stage of development are well illustrated by Samuelsson (16) for *Andromeda polifolia* in his Figure 3g. These central cells continue to multiply and enlarge, developing into a multicellular endosperm formed of elongated, very thin-walled cells. Even after these attain their maximum size, they have very little cell content. These thin-walled cells are the endosperm tissue immediately prior to the deposition of reserve food (Fig. 19).

(*b*) *Haustoria*

The description starts again at the eight-celled stage of the endosperm (Fig. 10).

Further development of the two terminal cells at each end is very different from that of four in the center. Both terminal pairs are richly protoplasmic but otherwise their initial development varies greatly. The micropylar pair separate (Fig. 10) and start to migrate into the micropylar haustorium. As this migration progresses the nuclei multiply very rapidly. As no mitotic figures were observed it is most likely that the nuclear divisions were amitotic. Samuelsson (16) suggested this possibility. Also, it is possible to observe all stages in what is apparently amitotic division. Figs. 12 and 13 illustrate a typical series. The migration of nuclei and protoplasm into a micropylar haustorium is illustrated in Fig. 11. The section is cut lateral to the zygote. The corresponding stage in the development of the chalazal haustorium is quite different. The two terminal cells at the chalazal end remain in contact and form an invading protoplasmic mass (Fig. 15). Again the nuclei multiply amitotically, but at first these nuclei remain in the embryo sac (Fig. 15), then they move rapidly to the invading ends of the cells. While moving from the embryo sac to the haustorium the nuclei multiply rapidly (Fig. 14). This migration of the nuclei is accompanied by a rapid enlargement of the haustorial cavity. The result is that the invading mass assumes a mushroom shape (Fig. 16) but by the end of this mid-stage development this haustorium is circular or oval in outline. The changes which close the mid-stage development are similar for both haustoria. For instance, both haustoria contain many free nuclei. Nine micropyle haustoria had an average of 10.5 nuclei and 12 chalazal haustoria an average of 7.1. In both haustoria these nuclei are imbedded in a densely granular protoplasmic mass, and the origin of the two haustoria is similar in that they each originated by the further development of two terminal cells of the eight nucleate endosperm.

(*c*) *New Structure Formed During Disintegration of Cells*

After the synergids and the tip of the pollen tube have started to disintegrate there appears at the micropyle end of the embryo sac a homogeneous plasma. This is at first conspicuous as an ever increasing sheath partially surrounding the zygote (Figs. 7, 8, 9, and 10). At first this plasma has imbedded in it many

black bodies similar to those mentioned previously (Fig. 17). These are usually globular and have the appearance of being a liquid suspended in a liquid. Both the plasma and the black inclusions appear to originate from the disintegrating structures at the micropyle end of the embryo sac (Fig. 18). Later the remains of the synergids, the end of the pollen tube, the black inclusions, and the other small black bodies all disappear, but the plasma increases in volume. A similar plasma may be found in the developing chalazal haustorium. There, it is conspicuous chiefly around the margin of the invading mass of protoplasm (Fig. 15). In the micropyle haustorium this plasma at one stage appears to surround the zygote completely, and is usually thicker on the micropyle side (Figs. 25 and 26). Transverse sections of this structure have various shapes (Figs. 25 and 26) and in addition this plasma forms strands or plates through all parts of the haustorium. These strands extend to the haustorial margin where they are attached (Fig. 27). Peltrisot (15) refers to these as "celluose traverses" and illustrates them for *Vaccinium uliginosum* in his Figure 5. By means of serial transverse sections the portion around the zygote may be traced from the center of the haustorium to the bounding cells on the micropyle side. There it is attached. This, of course, may be observed clearly in suitable longitudinal sections (Figs. 21, 23, and 28). It may be to what Artopoeus (1) refers when he reports that the young embryo is attached to a synergid. No attachment to a synergid was observed in the present investigation. When stained with orange G the plasma has an amber-like appearance, and with its encasing sheath around the zygote, and its plates or branches, it forms a very conspicuous part of the micropyle haustorium.

(*d*) *Zygote Development*

The initial stages in the development of the zygote cannot be correlated with any one stage in the development of the endosperm. In the comparative time of development of these two structures there was a great variation from embryo sac to embryo sac even in the same ovary but before the zygote became active some endosperm cells had always been formed. Many instances were observed where the zygote had not emerged from the plasma sheath although the endosperm had reached an advanced stage of development. Fig. 27 illustrates such a case. The first sign of zygote development is noticeable as an elongation which causes it to protrude from the plasma sheath. The zygote is now an oval, densely protoplasmic body usually with a vacuole at the micropyle end (Fig. 24). Then development must be very rapid for, although many ovaries were examined at this stage, the zygote was not observed half way across the haustorium but always either just emerging from the encasing sheath as illustrated in Fig. 24 or stretching right across the haustorium with its base in the plasma sheath and its tip in contact with the haustorium side of the endosperm plug (Fig. 23). The zygote then is undivided and its protoplasm is unsupported by any definite wall. In the sections on a slide it is always more or less collapsed. It was very difficult to locate the nucleus but frequently a nucleus-like body could be observed

either near the middle or at the tip. This is the condition of the zygote before it starts to penetrate the endosperm plug and many instances were noted where the zygote had not developed beyond that stage even though the ovary as a whole had continued to develop.

IV. Final Stages

(*a*) *Integumentary Layers*

(*i*) *General.*—In the later development of the integument, three layers must be considered separately, namely: the outermost layer or epidermis, the middle layers, and the innermost layer. The last is known as the "endothelium" or as the "integumentary tapetum".

(*ii*) *Outermost layer.*—The cell walls of the outermost layer or epidermis of the ovule become very much thickened (Fig. 19) and finally form the single layered seed coat (Fig. 37*a*).

(*iii*) *Middle layers.*—The middle layers develop in one of three different ways. First, in the maturing of normal ovules its cells break down (Fig. 19), then later they become crushed and in the ripe seed they are barely distinguishable (Fig. 37*b*). Second, the cells of this layer may become very strongly lignified and fill the inside of the ovule with a compact tissue of thick walled polyhedral cells. Third, when the middle layer cells remain mostly parenchymatous, they, the endothelium, and the embryo sac contents may all completely disintegrate.

(*iv*) *Innermost layer.*—The endothelial cells at first form the innermost layer of the integument and immediately surround the mature embryo sac (Figs. 6–10). The endothelium in this early stage has been well illustrated by many investigators. As the endosperm enlarges the endothelial cells become elongated in a direction parallel to the long axis of the ovule. At first this change is very gradual (Fig. 11) but finally large plate-like cells are formed with slightly thickened walls, conspicuous peripheral cytoplasm, and very large nuclei (Fig. 35). In transverse view these cells have very little thickness (Fig. 11). At first this layer completely surrounds the endosperm (Fig. 20) and is continuous with the thickened layer around each haustorium (Fig. 35). As the endosperm enlarges, the part of this layer in the center breaks down into separate fragments, but even in the nearly mature ovule some of these cells may be identified immediately adjacent to the haustoria. Just before this layer breaks down it may be identified as a thin black line just outside the endosperm (Fig. 19). In ovules in which the embryo sac contents have disintegrated but the middle integumentary layers have remained parenchymatous, the integumentary tapetum persists for a time as two rows of disintegrating cells connecting the two haustoria (Fig. 36). An integumentary tapetum like that just described for the blueberry has been described by Hewitt (8) for *Lobelia amoene*, but in the blueberry the tapetum persists for a longer period since Hewitt reports that in *Lobelia amoene* "the tapetum reaches its maximum development about the time of fertilization" whereas in the blueberry the tapetum reaches its maximum development

shortly before the proembryo starts to grow into the endosperm. In the fully mature ovule the integumentary tapetum has so completely disintegrated that it cannot be identified.

(*b*) *Proembryo and Embryo*

After the elongated zygote has penetrated the endosperm plug at the entrance to the micropyle haustorium and starts to grow into the endosperm tissue there is nothing unusual in its method of development (when it does develop) or in its final structure. The first divisions are transverse, producing three or four densely protoplasmic cells at the apex and a suspensor (Fig. 28). Hence this proembryo falls into the class which Johansen (10) calls "The Soland Type", which is in agreement with what Palser (14) reported for *Cassiope* and Chou (5) for *Gaultheria*. The first division parallel to the long axis of the suspensor is in the penultimate cell (Fig. 29). The subsequent development of the proembryo and the embryo follows so closely the type described and illustrated in almost every textbook covering this subject that there is no need to go into details here, but the various stages are illustrated in Figs. 20 to 34.

The description of proembryo development for the lowbush blueberry is complicated by the fact that the more or less familiar and healthy form of development is frequently arrested at some stage. For instance, it has already been noted that in many instances the zygote does not emerge from the plasma sheath. In addition many ovules were observed in which the micropylar haustorium contained an elongated zygote which stretched right across the haustorium but the cell contents were disintegrating (Fig. 26). In many of these cases the endosperm was well formed but no trace of an embryo could be found in this endosperm although perfect and continuous serial sections were available. Then in other cases the elongated zygote appeared to be meeting with difficulties in penetrating the endosperm plug at the constriction (Fig. 22). Further, when the suspensor had penetrated the plug, the suspensor cells very soon became crushed, suggesting that lateral pressure was being exerted by the cells of the endosperm plug. The start of this crushing is evident in Fig. 21. Also, in some ovules, the remains of the suspensor could be traced as merely a fine line through the plug (Fig. 33). Finally, there were many cases observed where no trace of the suspensor could be found in the endosperm plug even though the proembryo on one side and the haustorial contents on the other were both perfectly normal. Fig. 28 illustrates such a case. All this evidence suggests that the suspensor becomes crushed by the cells of the endosperm plug. If the basal cell of the suspensor functions as an absorber of nutrient, it certainly could not exercise this function for the benefit of the proembryo in this species. Another interesting observation is that the proembryo imbedded in the endosperm has frequently disintegrated (Fig. 21). The low percentage of perfect seeds noted for this species may be due to some or to all of these departures from normal healthy development.

(*c*) *The Seed*

(*i*) *Technique employed.*—As is usually the case in the study of mature seeds the seed coat made it practically impossible to cut satisfactory serial sections on a rotary microtome. To overcome this difficulty, at least partially, masses of seeds were placed in lens paper bags, imbedded in celloidin, and sectioned on the sliding microtome. In this way thousands of sections were obtained and suitable ones were selected from these, but it was not possible to obtain serial sections by this method. To obtain serial sections of the endosperm and embryo the seed coat was removed. This was done with the sharp rigid tungsten needles described by Bell and Facey (4). First, by a tangential cut with a razor blade, a small piece of seed coat was cut off from one side. Then, starting from this aperture, the remainder of the seed coat was snapped off with the needles. In this way an uninjured endosperm with enclosed embryo was obtained. It was possible to get serial sections of this structure on the rotary microtome.

(*ii*) *Seed types.*—Corresponding to the three types of development for the middle integumentary layers, there are chiefly three types of seed, namely: perfect large seeds, medium sized lignified seeds, and collapsed seeds. Each of these is described below.

(*iii*) *Perfect seeds.*—A perfect seed is illustrated in Fig. 37*a*. The very similar seed of *Vaccinium vitis-idaea* has been described by Peltrisot (15). In the blueberry the cells of the single layered seed coat are very heavily lignified on their inner and lateral walls. Simple pits are numerous and conspicuous (Fig. 37*b*). In this final stage both haustoria are very similar. In them the protoplasm has completely disintegrated into an opaque structureless granular mass and the plasma strands may stand out very clearly. These haustoria end up as hard brittle little lumps at each end of the endosperm. The endosperm is filled with reserve food (Fig. 37*b*) except in the vicinity of the cotyledons where there is evidence of absorption (Figs. 34 and 37*a*). The reserve food was deposited shortly before the stage illustrated in Fig. 33, that is, when the embryo is starting to enlarge within the endosperm. In the embryo the cotyledons and root cap are differentiated but the stem tip cannot be identified with certainty (Fig. 37*a*). As the embryo is straight and median the seed is classified by Martin (13) as "axile linear".

(*iv*) *Lignified seeds.*—One of the lignified seeds is illustrated in Fig. 38*a*. They can be identified quite easily if thoroughly soaked in a liquid such as alcohol or water and viewed with transmitted light. They are then comparatively transparent and golden brown in color. The middle layers of the integument form the main bulk of this seed and are composed of heavily lignified cells. The original location of the embryo sac is occupied by a row of brown thick-walled small cells (Fig. 38*b*). There is no embryo in this seed. It is usually medium in size but it may be large.

(*v*) *Collapsed seeds.*—The collapsed seeds are smaller than the others and assume various shapes as illustrated in Figs. 39 and 40. Most of the cells inside the seed coat are crushed and have completely disintegrated (Fig. 40).

A few of them may be lignified (Fig. 39). Sometimes there is a fairly large air space either at one end or occupying the whole inside of the seed. As a result some of these seeds will float when placed in water. There is no embryo in these seeds.

(*vi*) *Other types.*—The types listed above made up the bulk of the seeds but some other types occurred occasionally. For instance there were some with a complete endosperm of thin walled empty cells (Fig. 43). In others the empty endosperm cells were limited to one end. No embryo was ever found in such seeds. The most unusual seed observed was that illustrated in Fig. 42. This diagram is median longitudinal and through both haustoria. The endosperm was perfectly normal and was stocked with reserve food, yet there was no sign of an embryo. In the many hundreds of seeds cut in celloidin three seeds of this type were observed. Certainly if each of these seeds contained an embryo it was not the normal size or in the normal position, for as indicated by Figs. 37*a* and 41 a longitudinal section through both haustoria of an average seed would be bound to include some of the embryo. However, the occurrence of a seed with normal endosperm and no embryo has not been proved, for in every continuous series cut through the endosperm of a large seed an embryo was present. A seed with a complete endosperm and yet no embryo is not an impossibility however, for Kerr (11) has reported having found this type of seed in the blackberry. If it does occur in the blueberry it must be very rare.

Discussion

The most interesting thing about the facts presented in this article is the possible correlation between the low percentage of viable seeds and the many times the zygote and proembryo became inactivated during development. These occasions of inactivation start when the zygote does not emerge from the plasma sheath. Then other occasions when the proembryo appears to have difficulties are as follows: it may die before it leaves the haustorium, have difficulty in penetrating the endosperm plug, its suspensor within the endosperm may become crushed, and it frequently may die during its early stages of development within the endosperm. Sterility is a very complicated subject and an enormous amount of experimental work is always necessary before one can draw conclusions. As experimental work in this investigation was zero, no conclusions will be drawn, but cytologists as a rule associate sterility with hybridization. For instance Babcock and Clausen (2, p. 306) state: "In many instances interspecific hybridization gives only a very small percentage of the normal complement of seed". If this association of sterility and hybridization can be applied to the present case it is of interest in that it would suggest that *Vaccinium angustifolium* var. *laevifolium* is a hybrid.

Acknowledgments

The author was particularly fortunate throughout this work in being assisted by three graduates who during their undergraduate course had majored in Biology. They were Miss Jane Burchill, Mrs. Henry J. Presutti,

and Miss Elspeth C. Giffin. Miss Giffin drew all the figures. Again the author wishes to express his indebtedness to Dalhousie University where laboratory space was provided for this work.

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EXPLANATION OF FIGURES

FIGS. 1–19. All drawings and diagrams were done with the aid of a camera lucida. The homogeneous plasma, when present, is lined stippled.

FIG. 1. Median l.s. of ovule in which embryo sac contents have disintegrated and nucellar cells have remained alive. (×300)

FIGS. 2–4. Stages in triple fusion. In all these, one male cell is distinct in cytoplasm of egg.

FIG. 2. A male cell beside two polar nuclei. (×230)

FIG. 3. Cytoplasm of two polar nuclei and male cell starting to fuse. (×230)

FIG. 4. Formation of primary endosperm nucleus complete. (×230)

FIGS. 5–10. Early stages in endosperm formation.

FIG. 5. Division of primary endosperm nucleus. (×230)

FIG. 6. Two endosperm nuclei. No wall between them. (×230)

FIG. 7. Two endosperm nuclei, micropylar one dividing. (×155)

FIG. 8. Four endosperm nuclei. No walls between them. (×230)

FIG. 9. Four endosperm cells. Walls have been formed. (×155)

FIG. 10. Eight endosperm nuclei. Of the four central cells, only two are in plane of drawing. (×155)

FIG. 11. Endosperm nuclei migrating into micropylar haustorium. (×230)

FIGS. 12–14. Stages in amitosis.

FIG. 12. In lower nucleus the two nucleoli have separated but division of nucleoplasm has not started. Between the upper nucleus and the two nucleolate lower nucleus, the division of the nucleoplasm is almost complete. From micropylar haustorium. (×635)

FIG. 13. Two nuclei from a micropylar haustorium. Division of nucleoplasm just completed. (×635)

FIG. 14. Multinucleate protoplasm streaming into chalazal haustorium. In the lower part of stream there are three nuclei between which division is starting. (×635)

FIGS. 15–16. Early stages in formation of chalazal haustorium.

FIG. 15. Protoplasm is just starting to invade and destroy cells of ovule but nuclei of invading mass are still in embryo sac. (×230)
FIG. 16. Four nuclei have migrated into chalazal haustorium and protoplasm has assumed mushroom shape. (×300)
FIGS. 17–18. Formation of homogeneous plasma with inclusions.
FIG. 17. Surface view. The plasma and its inclusions obscure the zygote, which is at a slightly lower level. (×355)
FIG. 18. Edge view. This illustrates how plasma and its inclusions appear to originate from disintegrating cells. (×355)
FIG. 19. Median l.s. of ovule in mid-stage of development. Integumentary tapetum is reduced to single broken line and middle integumentary layers are disintegrating. (×50)

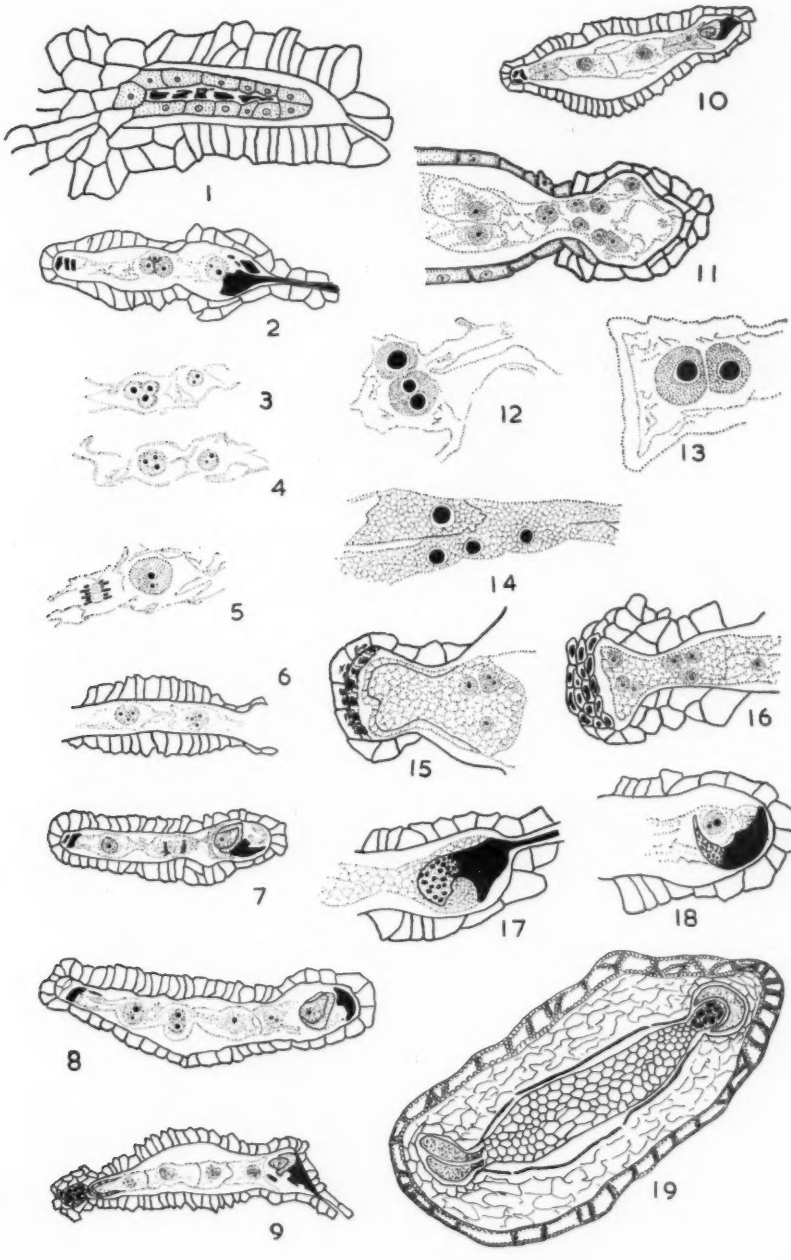
FIGS. 20–34. All drawings and diagrams were done with the aid of a camera lucida. The homogeneous plasma when present is lined stippled.

FIG. 20. T.s. through center of ovule at mid-stage of development. Integumentary tapetum completely surrounds endosperm. (×155)
FIGS. 21–23. Micropyle haustoria and portions of endosperm.
FIG. 21. Proembryo dead and suspensor partially crushed by cells of endosperm plug. (×230)
FIG. 22. Elongated zygote apparently meeting with difficulty in penetrating the endosperm plug. (×230)
FIG. 23. Elongated zygote stretching across haustorium. Zygote tip in contact with endosperm plug but not penetrating it. (×230)
FIG. 24. Initial elongation of zygote. (×635)
FIGS. 25–27. Transverse sections of plasma sheath surrounding zygote.
FIG. 25. A circular sheath. Protoplasm of zygote normal and living. (×635)
FIG. 26. A rhombic sheath. Protoplasm of zygote disintegrated. (×635)
FIG. 27. A sheath with lateral processes extending to wall of haustorium. (×635)
FIG. 28. Micropylar haustorium, portion of endosperm, and the proembryo. Proembryo divided by transverse walls only. No trace of suspensor in endosperm plug. (×230)
FIGS. 29–32. Stages in development of proembryo.
FIG. 29. Penultimate cell divided. (×300)
FIG. 30. Penultimate and apical cells divided. (×300)
FIG. 31. Penultimate, apical, and antipenultimate cells divided. (×300)
FIG. 32. Eight-celled stage. (×300)
FIGS. 33–34. Two stages in development of embryo.
FIG. 33. Embryo multicellular and spherical. (×300)
FIG. 34. Embryo with cotyledons differentiated. (×230)

FIGS. 35–43. All figures and diagrams were drawn with the aid of a camera lucida. Conventions used in diagrams: for endosperm, single diagonal cross hatching; for embryo, double diagonal cross hatching; for lignified tissue, squared cross hatching.

FIG. 35. Mature integumentary tapetum (endothelium) face view. Cells of lowermost layer oblique transverse. (×155)
FIG. 36. Integumentary tapetum from ovule in which the middle integumentary layers have remained parenchymatous and embryo sac contents have disintegrated. (×230)
FIG. 37*a*. L.s. of mature perfect seed. (×50)
FIG. 37*b*. Drawing of section from FIG. 37*a*. Endosperm cells filled with reserve food. (×300)
FIG. 38*a*. L.s. lignified seed. (×50)
FIG. 38*b*. Drawing of section from center of FIG. 38*a*. (×300)
FIG. 39. Small flattened seed. Some lignified cells in center. (×50)
FIG. 40. L.s. small completely collapsed seed. (×50)
FIG. 41. T.s. mature perfect seed. (×50)
FIG. 42. L.s. seed which in size and external appearance is exactly like a perfect seed but it has no embryo. (×50).
FIG. 43. Empty thin walled endosperm cells from seed which in size and external appearance is exactly like a lignified seed. (×300)

NOTE: Figs. 1–43 follow.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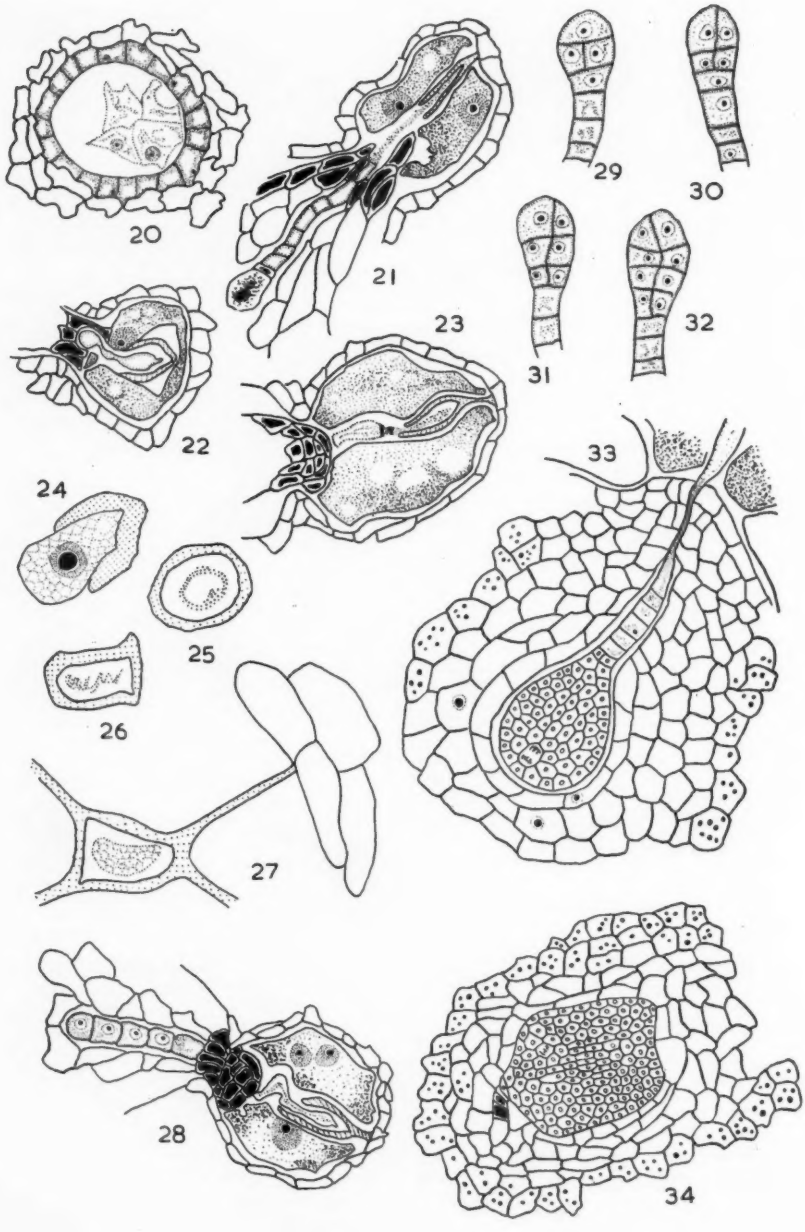
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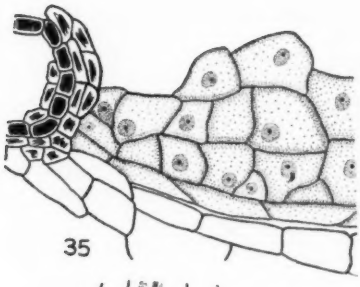

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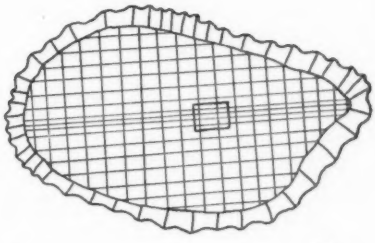

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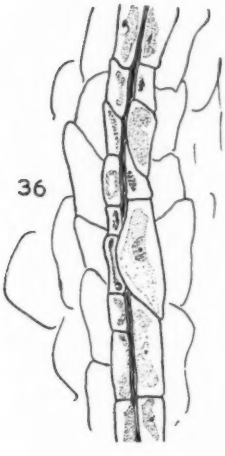

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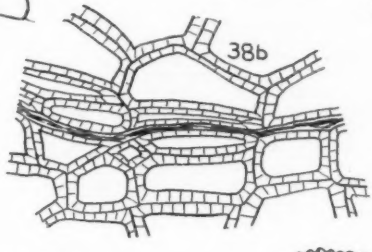

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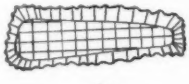

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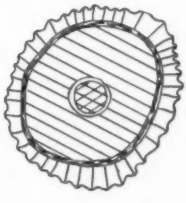

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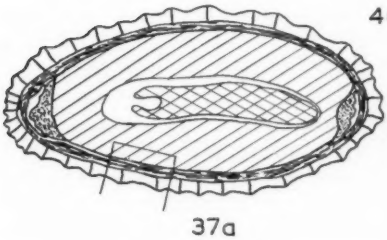

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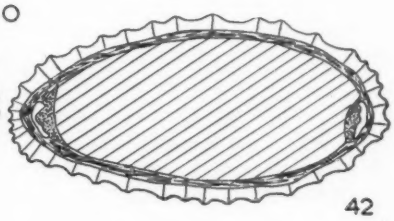

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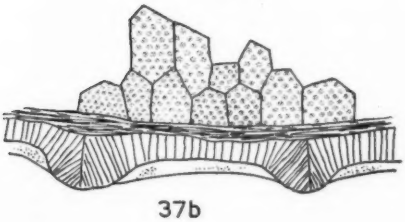

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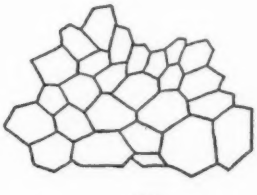

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THE DETERIORATION BY FUNGI OF JACK, RED, AND WHITE PINE KILLED BY FIRE IN ONTARIO[1]

J. T. BASHAM[2]

Abstract

The nature, causes, and rate of pathological deterioration of jack, red, and white pine killed by fire in the Mississagi region of Ontario in 1948 were studied to determine the practicability and probable duration of profitable salvage operations in such stands. Blue and brown sapwood stains appeared in all species 1 year after the fire, and became extensive during the succeeding 3 years. Sap rot was first noted 2 years after the fire; 5 years after the fire most of the sapwood was affected, and in some cases this rot extended into the heartwood. Three fungi, *Peniophora gigantea* (Fries) Massee, *Polyporus anceps* Peck, and *Polyporus abietinus* Dicks. ex Fries, were isolated consistently from white sap rots, while *Fomes pinicola* (Sw.) Cooke and *Fomes subroseus* (Weir) Overh. were recovered from many of the brown sap rots. The increase in the volume of heart rot encountered during the course of the study, chiefly associated with *Fomes pini* (Thore) Lloyd, was much greater than that observed in living pine over a similar period, and for this reason the excess was considered as a form of deterioration. The average rate of radial penetration of visible deterioration was significantly faster in trees with widely spaced annual rings in the outer portion of the bole than in slow-growing trees. Variations in the severity of burn in individual trees or stands apparently had little effect on the rate of pathological deterioration.

Introduction

On May 25, 1948, two fires were reported in the Mississagi–Chapleau area of Ontario. By the end of June, some 645,000 acres with forests composed mainly of valuable pine had been swept by the flames. Vast stands of dead, charred pine, spruce, and birch remained. In a brief period of 5 weeks a considerable portion of the dwindling reserves of white and red pine in the Province had been killed—but not destroyed. Forestry officials realized that in a few years these trees would be reduced to a worthless condition by various agents of deterioration. With this in mind, under government supervision, extensive salvage operations were begun in November, 1948. Practically all the cutting operations took place during the period 1948 to 1952, and over 300 million board feet of pine lumber was eventually recovered.

Although there have been a number of studies of the deterioration of fire-killed timber in North America (4, 6, 7, 8, 12, 13), none has dealt extensively with red and white pine. Members of the Laboratory of Forest Pathology, Maple, and the Forest Insect Laboratory, Sault Ste. Marie, as well as officers of the Department of Lands and Forests, Ontario, were eager to take advantage of this opportunity to collect information concerning the practicability and probable duration of profitable salvage operations in stands of this type. This report is based largely on data collected concerning the identity, frequency of occurrence, and mode of occurrence of fungi associated with the deterioration process in fire-killed jack, red, and white pine trees.

[1]Manuscript received in original form February 28, 1956, and, as revised, October 29, 1956. Contribution No. 335 from the Division of Forest Biology, Science Service, Department of Agriculture, Ottawa.

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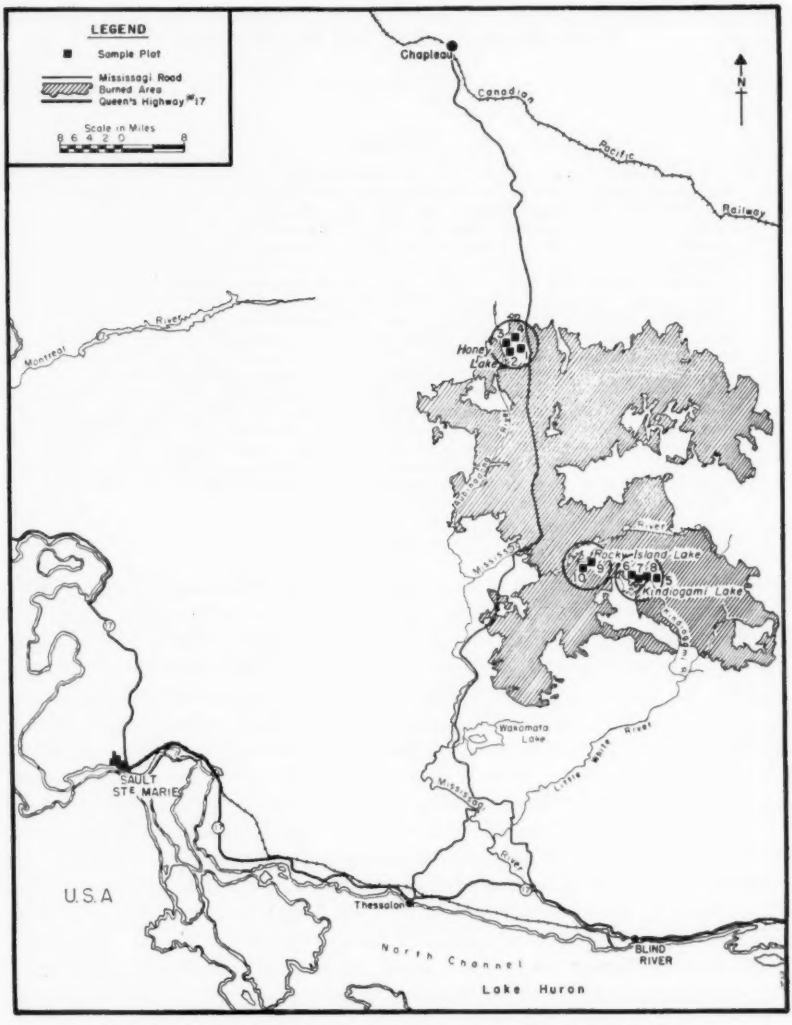

FIG. 1. Map of burned area illustrating the location of sample plots.

FIG. 2. View of severely burned jack pine stand, Honey Lake, taken in 1950.
FIG. 3. View of lightly burned red pine stand, Lake Kindiogami, taken in 1950.
FIG. 4. View of severely burned red pine stand, Lake Kindiogami, taken in 1950.
FIG. 5. Lightly burned jack pine log with bark intact and very lightly charred.
FIG. 6. Severely burned jack pine log with bark removed and some sapwood consumed.
FIG. 7. View of lightly burned jack pine stand, Honey Lake, taken in 1950. Living trees are scattered throughout the stand.

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Location of Work

The approximate area in which tree mortality occurred in the 1948 Mississagi fire is illustrated in Fig. 1. Sample plots, usually one or two acres in size, were established in jack pine and red pine stands of varying degrees of burn. The location of these plots is shown in Fig. 1. Representative areas of these plots are illustrated in Figs. 2 to 7. The severity of burn was judged on the basis of the ratio of surviving and dead trees, the extent of charring and loosening of the bark, the condition of the branches, and the presence or absence of the discolored foliage of killed trees.

Method of Study

Field Procedure

In the year following the fire, information concerning the extent of sap stain in red and white pine trees was obtained from individual trees dissected by officers of the Forest Insect Laboratory.

By 1950 the recoverable merchantable volume of the killed pine trees began to decrease owing to the appearance of rot in the sapwood. Permanent sample plots were established at that time and were designed so that one-quarter of each might be examined in the four successive field seasons from 1950 to 1953.

Each of the merchantable pine trees on these plots was felled, dissected, and described as thoroughly as possible on logarithmic tree measurement forms. The degree of burn of each tree, based on visible characteristics, was noted, and the total merchantable volume before the fire was computed in accordance with the Ontario Manual of Scaling Instructions. In addition, the volumes of heart rot, sap rot, sap stain, and consumed wood were plotted as accurately as possible for each tree. Samples of the stains and rots encountered were shipped to the Maple Laboratory where cultures were made which were sent to the Botany and Plant Pathology Division, Ottawa, for the identification of fungi associated with the several defects.

Office Tabulations

The total volume and the total merchantable volume in cubic feet were computed for each tree from the areas representing these volumes on the logarithmic tree measurement forms. Similarly, the portions of the total merchantable volume occupied by heart rot, sap rot, sap stain, and consumed wood were determined. The trees were scaled once again, deductions being made for heart rot and deterioration in accordance with the Ontario Scaling Manual.

The average depth of radial penetration of visible deterioration was computed for each tree. An analysis was made to determine whether any relationship existed between the depth of pathological deterioration and (*a*) tree diameter, (*b*) the average width of annual rings in the outer portion of the bole, and (*c*) the degree of burn.

TABLE I

DESCRIPTION OF SAMPLE PLOTS

Plot No. (1)	Predominant merch. species (2)	Location (3)	Plot area, ac. (4)	Plot established (5)	Pine trees of merchantable size: Jack pine (6)	Red pine (7)	White pine (8)	Total (9)	Av. D.B.H.* of merch. pine trees (10)	Age class at time of burn (11)	Est. degree of burn (12)
1	Jack pine	Honey L.	2.0	1950	96	0	25	121	13.4	150–160	Heavy
2	Jack pine	Honey L.	2.0	1950	206	0	23	229	12.3	150–160	Medium
3	Jack pine	Honey L.	1.75	1950	118	0	4	122	11.9	150–160	Heavy
4	Jack pine	Honey L.	2.50	1950	219	0	1	220	10.3	70– 80	Light
5	Red pine	L. Kindiogami	0.50	1950	3	41	3	47	15.3	140–150	Heavy
6	Red pine	L. Kindiogami	2.0	1950	5	158	49	212	14.2	140–150	Medium
7	Red pine	L. Kindiogami	1.25	1950	2	100	12	114	12.2	140–150	Light
8	Red pine	L. Kindiogami	0.75	1951	0	83	9	92	13.1	140–150	Medium
9	Red pine	Rocky Island L.	1.0	1952	5	78	22	105	14.9	140–150	Medium
10	Red pine	Rocky Island L.	1.0	1952	18	55	18	91	13.8	140–150	Heavy
Total			14.75		672	515	166	1,353			

*Diameter at breast height, outside bark.

TABLE II

THE ANNUAL FREQUENCY OF OCCURRENCE OF THE FIVE MAJOR TYPES OF FUNGAL DETERIORATION IN FIRE-KILLED JACK, RED, AND WHITE PINE

	Percentage of trees affected											
	Jack pine, 658 trees				Red pine, 462 trees				White pine, 156 trees			
Type of deterioration	1950	1951	1952	1953	1950	1951	1952	1953	1950	1951	1952	1953
Blue stain	90.2	93.1	82.2	12.3	100.0	100.0	100.0	25.5	96.2	97.1	85.4	8.3
Brown stain	38.2	64.4	64.4	5.6	95.8	89.0	58.8	11.1	84.6	85.3	47.9	6.3
Orange ring stain	34.3	33.9	41.5	51.9	1.0	0.0	1.5	0.0	3.8	0.0	43.8	18.8
White sap rot	34.8	55.2	80.5	95.1	36.4	65.9	100.0	98.7	38.4	41.2	95.8	100.0
Brown sap rot	33.8	42.5	65.3	66.7	44.8	50.0	63.4	71.2	23.1	47.1	66.7	75.0

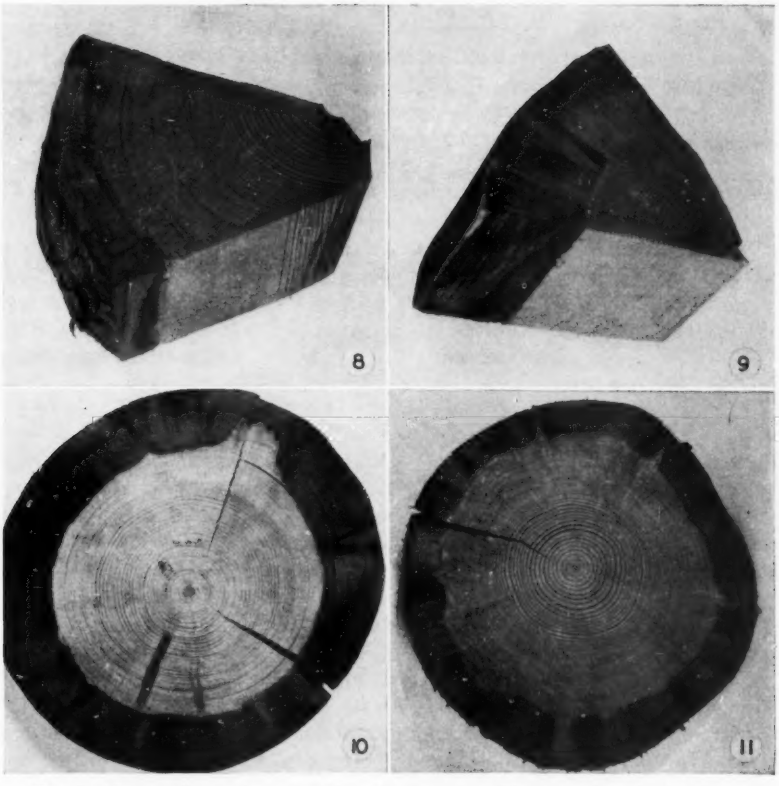

FIG. 8. Orange-ring stain at junction of sapwood and heartwood in dead jack pine, 2 years after the fire.
FIG. 9. Blue sap stain in dead jack pine, 2 years after the fire.
FIG. 10. Blue and brown stained sapwood in a transverse section of red pine tree, 2 years after the fire.
FIG. 11. Incipient sap rot and stained sapwood in a transverse section of red pine tree, 3 years after the fire.

Causes of Tree Deterioration

Immediately following even a severe fire, the reduction in the net merchantable volume of the stand is negligible. The killed trees remain suitable for the production of lumber and pulpwood with little or no reduction of grade until the agents of deterioration operate to reduce progressively the yield and quality of timber. Besides the slight immediate destruction of merchantable wood by the fire itself, the other deteriorating agents are fungal decay, insect attack, mechanical wear, and the destructive effects of exposure to all types of weather. Excessive breakage due directly to the fire may cause appreciable losses; apart from this one can disregard mechanical wear. The adverse effects of weather are of little significance compared to those of fungi and insects.

Evidence from other investigations indicates that in stands of this type, there is a close association between the two principal agents of deterioration, i.e. insects and fungi (9). In most cases, insect activity precedes fungal attack, and the insects may play an important role in distributing the fungi to the host trees. Although most insect activity terminates within 1 or 2 years following the death of the tree, fungal deterioration often continues until the entire trunk is destroyed.

Fungal Deterioration

Sap Stain and Sap Rot

After death, the sapwood, previously capable of resisting the invasion of harmful fungi, becomes exceedingly susceptible to sap-staining and sap-rotting organisms. These fungi enter the outer bole using the many avenues through the bark present in dead trees; as a rule they advance through the wood until, eventually, almost all of the tree is infected. This process takes a few to many years, depending upon various factors and conditions. In the interval between the death of the trees and complete destruction, decreasing amounts of perfectly sound, usable wood remain in the central portions of the boles. In many cases this wood can be profitably utilized for lumber or pulpwood.

The fungi involved in this type of deterioration of fire-killed pine fall into two distinct groups, both taxonomically and according to the type of deterioration with which they are associated. Those fungi responsible for sap stains belong mainly to the group Fungi Imperfecti; those also causing sap rots are members of the Class Basidiomyceteae, order Agaricales.

Three different sap stains, designated as blue stain, brown stain, and orange ring stain, and two types of sap rot, white and brown, were encountered in the fire-killed pine trees. Table II shows the annual volumetric occurrence of these five types of fungal deterioration in jack, red, and white pine, from 2 to 5 years after the fire.

Sap Stains

The three sap stains are illustrated in Figs. 8 to 11. Three fungi, not yet identified, were consistently isolated from wood affected by these three stains. These organisms are limited to the sapwood and can exist only from 1 to 3

years following the death of the trees because of their dependence upon a relatively high moisture content of the wood and upon the cell sap for nourishment (10).

Blue stain was unquestionably the most extensive stain encountered (1). Most of the sapwood of all three species exhibited blue stain 3 years after the fire. Many cases of brown stain were noted in the sapwood of trees examined after they had been dead for 1 year. These appeared usually as patches in the rings of blue stain, although occasionally the situation was reversed and a complete band of brown stain containing patches of blue stain occurred. It is possible that some of these brown stains were actually incipient stages of brown cubical sap rots. Boyce (3) states "brown sap stain . . . is confined to sapwood and is common on jack . . . and red pines, and . . . occasionally on . . . western white pines." Most of the organisms isolated from brown stained wood were identified as Fungi Imperfecti, and therefore this type of deterioration was classified as stain rather than as incipient rot.

The peculiar orange-ring stain observed in approximately half of the jack pine trees and in many white pine trees is of little economic importance. The ring, roughly one-quarter of an inch wide, occurs at or close to the dividing line between heartwood and sapwood, and was commonly observed in association with tunnels of *Monochamus* spp. wood borers. Although it has been definitely ascertained that this stain is caused by a species of the Fungi Imperfecti group, its obvious departure from the usual mode of occurrence of sap staining fungi makes laboratory inoculation tests desirable.

Sap Rots

White and brown sap rots of fire-killed pine are illustrated in Figs. 12 to 15. These decays result in reductions in the strength properties of infected wood, and may ultimately destroy both the sapwood and heartwood. The "white rot" group of fungi leave a residue mostly of cellulose, whereas the "brown rot" organisms leave the lignin practically unaffected.

The determination of the causal organisms of these sap rots was complicated by the fact that in many instances in 1950 and 1951 they were masked in culture by the sap staining fungi with which they grew in close association. Furthermore, sap rots were often difficult to trace because of their complex, patchy occurrence. Nevertheless, it is felt that the frequency with which various fungi were isolated from samples of sap rot, as shown in Table III, indicates the relative importance of these fungi in causing sap rot in fire-killed jack, red, and white pine.

FIG. 12. Patches of brown cubical sap rot caused by *Fomes pinicola* in dead jack pine, 2 years after the fire.

FIG. 13. White stringy and brown cubical sap rot in the outer sapwood in transverse section of a dead red pine tree, 3 years after the fire.

FIG. 14. White stringy sap rot in entire sapwood portion of a jack pine tree, transverse section, 4 years after the fire.

FIG. 15. Brown cubical sap rot in entire sapwood portion of a red pine tree, transverse section, 5 years after the fire.

FIG. 16. *Fomes pini* heart rot in a red pine tree, transverse section, 3 years after the fire.

FIG. 17. *Fomes pini* heart rot in a white pine tree, radial section.

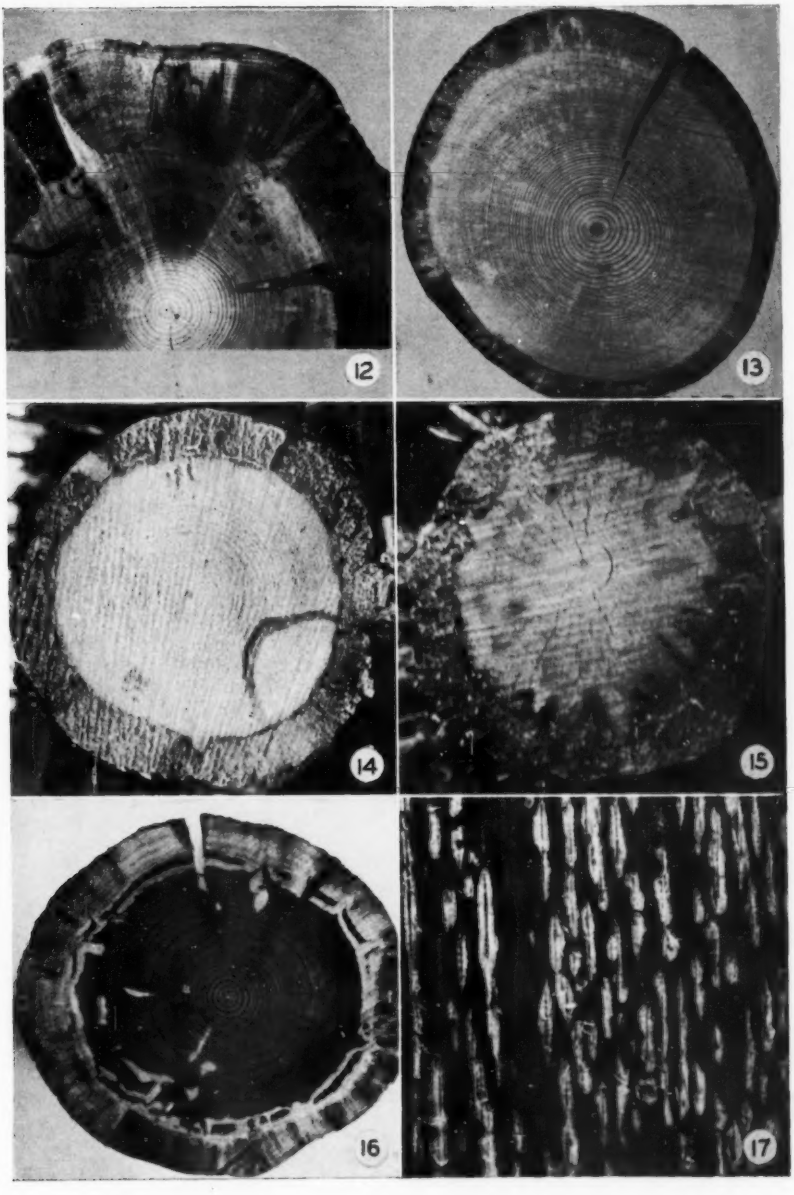
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TABLE III

FUNGI ISOLATED FROM SAP ROT IN FIRE-KILLED PINE TREES, AND THEIR FREQUENCY OF OCCURRENCE

	Total No. isolations	Annual percentages of isolations from white rots and brown rots											
		Jack pine				Red pine				White pine			
		1950	1951	1952	1953	1950	1951	1952	1953	1950	1951	1952	1953
"White" sap rots													
Peniophora gigantea (Fries) Massee	170	14.8	35.6	34.6	14.3	65.0	69.2	40.0	33.9	60.0	9.1	8.3	17.6
Polyporus anceps Peck	135	11.1	45.7	44.3	30.6		11.5	25.7	18.7		45.4	29.2	23.5
Polyporus abietinus Dicks. ex Fries	104	59.3	5.1	11.5	22.5	20.0	7.7	18.0	16.1	40.0	36.4	54.2	35.3
Corticium galactinum (Fries) Burt	35				12.3			9.5	13.4			8.3	11.8
Poria subacida (Peck) Sacc.	14	11.1	1.7	3.9	4.1	10.0	3.9		1.8				5.9
Phlebia merismoides Fries	14	3.7		1.9				3.8	7.1				
Peniophora aspera (Pers.) Sacc.	5				2.0			1.0	2.7				
Trechispora brinkmanni (Bres.) Rog. & Jacks.	5				2.0	5.0	7.7		0.9				
Peniophora cinerea (Pers. ex Fries) Cooke	4		3.4		2.0						9.1		
Polyporus volvatus Peck	4							1.0	2.7				
Odonia bicolor (Fries) Bres.	3			1.9	4.1								
Polyporus dichrous Fries	2				2.0				0.9				
Polyporus borealis Fries	2								0.9				5.9
Claudopus nidulans Fries	2			1.9				1.0					
Poria rufa (Schrad. ex Fries) Cooke	1				2.0								
Poria notata Overh.	1				2.0								
Polyporus albellus Peck	1								0.9				
Total	502												
"Brown" sap rots													
Fomes pinicola (Sw.) Cooke	123	80.9	66.0	36.4	9.1	100.0	75.0	29.5	17.9	100.0	85.7	72.7	
Fomes subroseus (Weir) Overh.	39			6.1	22.7				74.4				75.0
Coniophora puteana (Schum. ex Fries) Karst.	38	4.8	10.0	24.2	13.6			29.5	5.1			9.1	
Lenzites saepiaria Wulf. ex Fries	32	9.5	18.0	27.3	18.2			9.8	2.6				25.0
Poria monticola Murr.	24	4.8	4.0	3.0			25.0	21.3			14.3	18.2	
Merulius himantioides Fries	11		2.0		22.7			8.2					
Peniophora phlebioides Jacks. & Dearden	2				9.1								
Corticium fuscostratum Burt	1			3.0									
Poria sinuosa (Fries) Cooke	1							1.7					
Polyporus gutulatus Peck	1				4.6								
Total	272												

From Table III it is apparent that of the 17 organisms isolated from white sap rots, three fungi, *Peniophora gigantea*, *Polyporus anceps*, and *Polyporus abietinus*, accounted for over 80% of the isolations. Each of these fungi has previously been reported to be associated with the deterioration of coniferous trees. Spaulding and Hansbrough (14) isolated all three from balsam fir, hemlock, and white, red, and pitch pine slash. Cartwright and Findlay (5) report that *P. gigantea* is responsible for a pale yellowish-brown rot in the sapwood of coniferous logs in Great Britain. In a study of the deterioration of balsam fir in Ontario (2) it was found that most of the advanced deterioration was attributable to *P. abietinus*, although *P. gigantea* and *P. anceps* were occasionally isolated from decayed sapwood. Differences in the appearance of sapwood rotted by these three fungi were so slight that they could not be distinguished except by personnel who had handled identified samples of these rots for a considerable length of time. The small, whitish fruiting bodies of *P. abietinus* are frequently seen on coniferous trees which have been dead for from 2 to 5 years; however, fructifications of *P. gigantea* and *P. anceps* are relatively rare. This may explain why the latter two fungi are as a rule regarded as of less importance than *P. abietinus* in the deterioration of dead coniferous trees and slash.

Corticium galactinum (Fries) Burt and *Poria subacida* (Peck) Sacc., isolated 35 and 14 times respectively, are better known as the causes of white butt and root rots of coniferous trees. *Phlebia merismoides* Fries, isolated 14 times, has seldom been recorded in association with decayed wood. The remaining 11 organisms obtained from white sap rots were isolated five times or less, and are presumably of minor significance in the deterioration process of fire-killed jack, red, and white pine.

Table III shows that *Fomes pinicola* was the most frequently encountered brown sap rot, accounting for almost half of the identified isolations made from this type of decay. *F. pinicola* is a very common cause of brown sap rot in both coniferous and broad-leaved trees in North America, and is occasionally found associated with brown heart rot of living conifers.

Five other organisms were isolated more than 10 times from the brown sap rots of fire-killed pine. Two of these, *Fomes subroseus* (Weir) Overh. (39 isolations) and *Lenzites saepiaria* Wulf. ex Fries (32 isolations) are known to occur frequently as brown sap rots of dead coniferous trees. The remaining three fungi, *Coniophora puteana* (Schum. ex Fries) Karst. (38 isolations), *Poria monticola* Murr. (24 isolations), and *Merulius himantioides* Fries (11 isolations), are probably better known as the causes of brown cubical heart rots in living trees, although the author has reported *C. puteana* as the cause of brown sap rot of balsam fir killed by spruce budworm in Ontario (2) and *P. monticola* is known to be of considerable importance as a destroyer of timber in service. The five isolations made of the four remaining fungi from brown sap rots are presumably of very little significance.

A close examination of Table III reveals two apparent instances of changes in the relative frequency of occurrence of the more commonly isolated sap rot fungi during the 4 years in which these isolations were made. The first

of these concerns the occurrence of *P. anceps*. Table III shows that this organism was isolated quite frequently from all three species of pine in 1951, 1952, and 1953; however, in 1950, isolations were limited to jack pine and comprised only 11% of the white sap rot isolations made from this species in that year. The second and more striking instance is the replacement of *F. pinicola* by *F. subroseus* in the brown sap rot complex of all three host species. *F. subroseus* was isolated most frequently from the brown sap rots of jack, red, and white pine in 1953; however, it was not encountered at all during the first 2 years of this study.

Heart Rot

The rate of progress of heartwood decay in living trees varies, depending upon the specific fungus and host tree involved. Most investigations that have been carried out report a rate of about 5 inches annually in a longitudinal direction (15). Changes in the extent of heart rot, during the period in which the deterioration of dead trees is of economic importance, have been generally disregarded since it was assumed that the losses resulting from this extension were insignificant in comparison with those caused by other agents of deterioration.

All but one of the 10 sample plots in this study were established in 140- or 160-year-old even-aged pine stands. Unpublished data collected in conjunction with the Department of Lands and Forests cull survey project indicate that, for jack pine in northern Ontario, the percentage of the total merchantable volume deducted because of heart rot increases from 17 to 21% over the 5-year period from 147 to 152 years of age. These figures are based on a sample of 1500 jack pine trees from several regions of Ontario, including the Chapleau district. White (16), in a study of the decay relationships of white pine in the Temagami Lake and Ottawa Valley areas, found that in this age class the percentage of the total merchantable volume deducted because of heart rot increased from 34.7 to 35.1% over a similar 5-year period. Although no comparable data is available for red pine, it would seem from the foregoing that the increase in heart rot volume in living red pine of this age group during a 5-year period would be quite small.

The unanticipated but pronounced increase in heart rot deductions that occurred in fire-killed jack, red, and white pine during the 5-year interval following the fire is shown clearly in Fig. 18. The consistent increase observed in all three species is significantly higher than one would expect to find in living trees over the same period. It is postulated that changes in the heartwood caused by the death of these trees, possibly an increase in the average wood temperatures, have in some manner provided a more suitable substrate for the heart rot fungi, thereby stimulating them to much faster growth, colonization, and destruction of the wood than occurs normally in living trees.

It seems reasonable, under these circumstances, that this abnormal increase in the rate of progress of heart rots should be included as a form of deterioration of these killed trees. It was assumed from the shape of the curves in Fig. 18 that the percentage of the total merchantable volume deducted because of

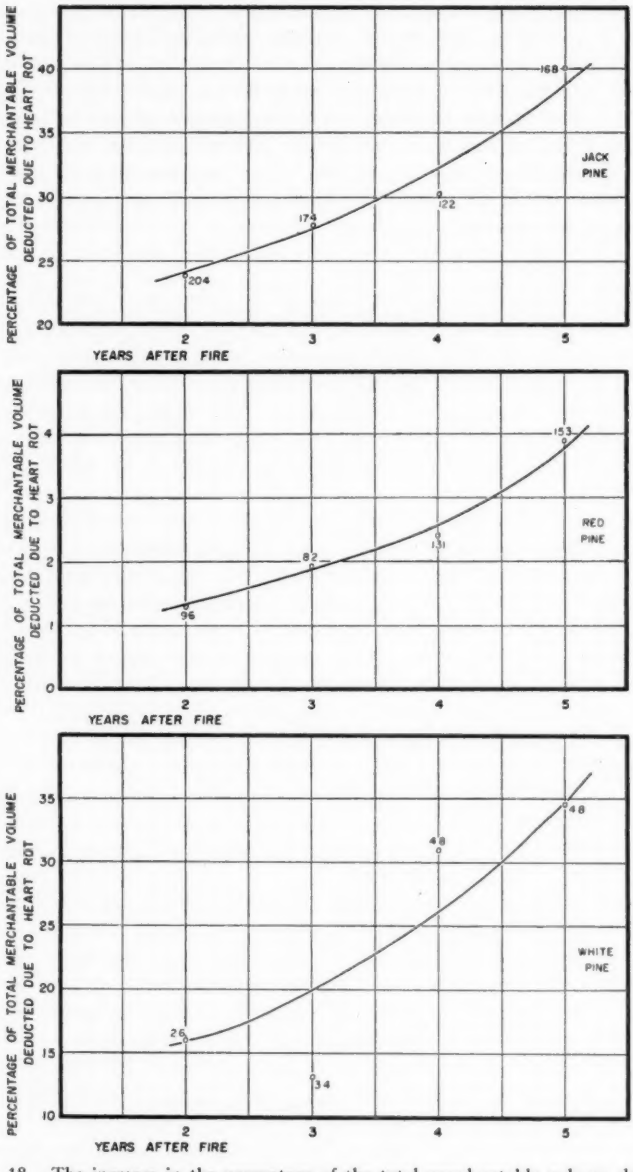

FIG. 18. The increase in the percentage of the total merchantable volume deducted because of heart rot in dead jack, red, and white pine during the 5-year period following the fire.

heart rot 2 years after the fire can be considered as approximately the same as that which would have been deducted at the time the trees were killed. The extent of heart rot deterioration can hence be estimated from Fig. 18 as the difference between the percentage of the total merchantable volume deducted because of heart rot 2 years after the fire and that deducted at the interval under consideration.

Practically all of this increase in heart rot volume occurred in the trunk rots of these trees. The increase in the volume of butt rots was very slight. One fungus, *Fomes pini*, was isolated from over 80% of the trunk rots of jack, red, and white pine. This form of deterioration, therefore, may be almost entirely attributed to the action of this organism. *Fomes pini* heart rot is illustrated in Figs. 16 and 17.

Factors That May Influence the Rate of Deterioration

Tree Growth Rate and Diameter

The radial growth in inches per decade at stump height for the last 6 decades before death was recorded for each tree. On the basis of these figures the trees were classified into three groups representing those with slow, medium, and fast growth in the sapwood and outer heartwood. Since the trees sampled occurred in even-aged stands, it follows that in general those trees with relatively fast growth over the last 60 years before death would tend to be of greater diameter than those with a relatively slow growth rate. Table IV indicates clearly that, in each of the three species of pine studied, a pronounced relationship exists between the average width of the annual rings in the outer bole and tree diameter.

Table IV also shows that the average depth of radial penetration of visible deterioration is greatest in those trees with widely-spaced annual rings in the outer bole, and least in those with narrowly-spaced rings. This relationship

TABLE IV

THE RELATIONSHIP BETWEEN THE WIDTH OF ANNUAL RINGS IN THE OUTER BOLE, TREE DIAMETER, AND THE DEPTH OF RADIAL PENETRATION OF VISIBLE DETERIORATION (R.P.D.)

		Rings narrowly spaced (slow growth)			Rings moderately spaced (medium growth)			Rings widely spaced (fast growth)		
Species (1)	No. years after fire (2)	No. trees (3)	Av. D.B.H. (4)	Av. depth R.P.D. (in.) (5)	No. trees (6)	Av. D.B.H. (7)	Av. depth R.P.D. (in.) (8)	No. trees (9)	Av. D.B.H. (10)	Av. depth R.P.D. (in.) (11)
Jack pine	2	37	11.8	0.78	39	12.1	1.14	24	14.0	1.59
Jack pine	3	24	10.1	1.13	38	12.7	1.43	23	14.6	1.77
Jack pine	4	35	11.7	1.24	35	12.5	1.78	21	14.3	2.09
Jack pine	5	18	11.9	1.65	40	12.3	2.02	30	13.4	2.12
Red pine	2	22	12.5	2.05	37	13.3	3.04	30	16.2	4.06
Red pine	3	37	12.7	2.39	32	13.8	3.00	6	14.6	3.60
Red pine	4	38	12.5	2.68	47	13.9	3.81	46	15.5	4.72
Red pine	5	24	12.7	3.03	48	13.3	3.52	45	14.0	4.52
White pine	2	6	12.6	1.27	3	15.4	1.44	5	16.8	2.52
White pine	3	5	13.7	2.08	7	13.2	1.49	11	13.5	2.18
White pine	4	11	14.4	2.19	11	14.4	2.75	16	15.9	3.39
White pine	5	14	12.9	2.06	8	16.1	2.85	4	18.5	3.90

TABLE V

THE RELATIONSHIP BETWEEN TREE DIAMETER, SAP ROT, AND SAP STAIN IN FIRE-KILLED JACK PINE AND RED PINE

	% total merchantable volume occupied by sap stain				% total merchantable volume deducted because of sap rot			
	Species				Species			
	Jack pine		Red pine		Jack pine		Red pine	
	Diameter classes*				Diameter classes*			
	9–11	13–18	9–12	14–21	9–11	13–18	9–12	14–21
Two years after fire	20.0	16.3	41.1	47.4	11.8	4.7	2.9	5.2
Three years after fire	26.2	20.9	38.7	40.6	15.3	17.5	18.3	14.6
Four years after fire	13.5	11.0	25.8	19.8	42.1	24.0	64.4	48.9
Five years after fire	4.3	0.3	6.0	7.2	68.0	24.4	85.5	68.0

*Diameter at breast height, outside bark.

is very pronounced in all three species of pine. A similar relationship was found by Kimmey and Furniss in an investigation of the deterioration of fire-killed Douglas fir (7). It is generally agreed (15) that fungi penetrate wood in which the annual rings are relatively close together at a slower rate than wood in which the rings are more widely separated. A logical explanation of this fact is found in a comparison of the number and thickness of cell walls that must be penetrated in slow-growing wood with the reduced number and thickness of those encountered in a similar distance in fast-growing wood.

It is possible that the thicker sapwood usually associated with fast growing trees provides an explanation of the relationship between growth rate and deterioration. Dead sapwood, because of its moist condition and relative lack of growth inhibiting substances, constitutes a more favorable environment than heartwood for the growth and establishment of fungi. Larger trees possess more widely-spaced annual rings and, as a rule, thicker sapwood than smaller trees of the same age class; radial penetration by deteriorating fungi is hence more rapid in the larger trees, as shown in Table IV.

The relationship between jack pine and red pine tree diameters, scaling deductions directly due to sap rot, and sap stain volume is presented in Table V. This table shows that tree size had no significant effect upon these last two factors until a period of 4 years had elapsed after the fire; from this time on, a larger percentage of sap stained volume and greater sap rot deductions occur in the smaller than in the larger trees, despite the faster rate of penetration of deteriorating fungi in the latter. The reason for this is that the greater volume of deterioration in larger trees is more than compensated for by the much greater volume of sound, merchantable wood recoverable from large trees compared to that recoverable from small trees 4 or 5 years after the fire.

The Degree of Burn

In Table VI the jack pine and red pine trees have been classified according to the degree of burn based on the condition and appearance of the trunk and crown. In cases where the trunk was lightly to moderately charred with

TABLE VI

THE RELATIONSHIP BETWEEN THE SEVERITY OF BURN AND THE DEPTH OF RADIAL PENETRATION OF DETERIORATION IN FIRE-KILLED JACK PINE AND RED PINE

Species (1)	No. years after fire (2)	Av. depth of radial penetration of deterioration (in.)		
		Lightly burned trees (3)	Moderately burned trees (4)	Severely burned trees (5)
Jack pine	2	1.0	1.1	1.4
Jack pine	3	1.9	1.6	1.4
Jack pine	4	1.8	1.7	1.6
Jack pine	5	2.6	2.5	2.1
Red pine	2	3.0	3.4	2.8
Red pine	3	2.7	2.9	2.6
Red pine	4	3.5	3.8	4.0
Red pine	5	3.8	4.0	3.9

bark still intact, the branches relatively undamaged, and many dead needles retained, the trees were considered lightly burned. Trees with trunk and branches heavily charred, the bark entirely or almost entirely removed, and no foliage remaining, were classed as severely burned. Those trees with medium burn characteristics that could not be placed in either of the above two categories were regarded as moderately burned.

The average depth of radial penetration of visible deterioration of lightly, moderately, and severely burned jack pine and red pine at various periods after the fire is presented in Table VI, and is shown graphically in Fig. 19. It is evident that there is no consistent difference in the rate of deterioration of jack pine and red pine that have been subjected to various degrees of burn. From 3 to 5 years following the fire, lightly burned jack pine appear to deteriorate slightly faster than moderately burned trees, which in turn deteriorate slightly faster than severely burned trees. With the red pine the situation is reversed, and lightly burned trees have the slowest rate of deterioration.

In all cases, the differences in the average depth of visible deterioration among trees of varying degree of burn were very small, and not statistically significant. It is considered likely that the apparent differences in the rate of deterioration in lightly, moderately, and severely burned trees may be accounted for by chance alone, and that the degree of burn actually had very little or no effect upon this rate.

In previous investigations of the deterioration of fire-killed trees, fairly conclusive evidence has been developed to indicate that the amount and rate of deterioration tends to decrease as the severity of burn increases. The explanation given is that a light burn often leaves the bark intact on the tree whereas a heavy burn removes it, with the result that lightly burned trees possess moister wood which provides more favorable conditions for the growth and establishment of deteriorating fungi. One possible reason why this

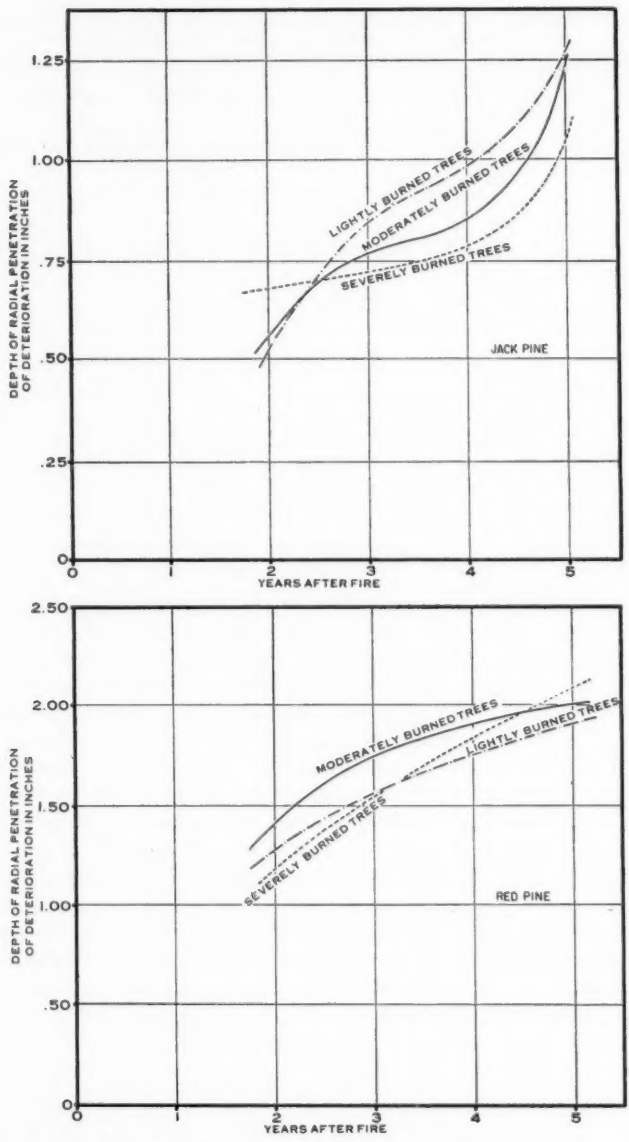

FIG. 19. The effect of the severity of burn of fire-killed jack pine and red pine upon the rate of radial penetration of visible fungal deterioration.

relationship does not exist in jack pine and red pine killed in the Mississagi fire is that the lightly and severely burned trees of this investigation were not as divergent in degree of burn as those encountered in other studies.

The Deterioration of Jack, Red, and White Pine Trees

Thirty-four white pine and 59 red pine were examined for pathological deterioration at Lake Kindiogami 1 year after the fire. It soon became apparent that the only type of pathological deterioration present at that time was a blue sapwood stain. Observations made of a few representative jack pine indicated that the same condition existed in this species. On the basis of the uniformity observed in these trees, it was decided that this sample would provide an adequate estimate of the amount of blue stain to be expected in red and white pine approximately 1 year after the fire.

Blue stain was found in 88.2% of the white pine and 74.6% of the red pine examined, usually in the form of patches or streaks in the outer sapwood. The average depth of radial penetration of blue stain was 0.9 in. in white pine and 0.4 in. in red pine. The more extensive blue stain in white pine than in red pine at this time, together with the greater insect damage that was observed in the former species, corroborates the results of the mill study conducted by Prebble (11).

The changes that occurred in the actual volumes of heart rot and of various kinds of deterioration in fire-killed jack, red, and white pine from 2 to 5 years following the death of the trees are summarized in Table VII. It is evident from this table that the progress of deterioration follows the same general pattern in all three species. The only noticeable differences are in the volumes of heart rot and in the volumes of wood destroyed by the fire.

As the interval following the fire lengthened, the volume of heart rot in the trees increased at a progressively greater rate. Sap stain, but no sap rot, occurred in the sapwood 1 year after the fire. One year later most of the sapwood was stained. The volume of wood occupied by sap stain increased very little, if at all, after that time. The reason for this is probably that the sap stain organisms remain viable only in relatively moist wood which contains the remnants of the cell contents.

A small proportion of the volume of each species was affected by sap rot 2 years after the fire. It occurred at this time in the form of small patches in the outer sapwood. In succeeding years, sapwood deterioration consisted of the continuous replacement of existing sap stain by sap rot. Very little sap stain remained in these trees 5 years after the fire and a few trees with sap rot extending short distances into the heartwood were encountered at this time.

The progressive increase in the percentage of the merchantable volume occupied by sap rot following the fire is the most significant factor in the reduction of the recoverable merchantable volume of the fire-killed jack, red, and white pine. Fig. 20 has been constructed to provide a direct comparison of this factor among these three species.

TABLE VII

ACTUAL VOLUMES OF HEART ROT AND OF VARIOUS KINDS OF DETERIORATION IN DEAD JACK, RED, AND WHITE PINE AT DIFFERENT INTERVALS FOLLOWING THE FIRE

Species (1)	No. years after fire (2)	No. killed trees (3)	Av. D.B.H. (4)	Total vol. trees (cu. ft.) (5)	Total merch. vol. (cu. ft.) (6)	% merch. vol. occupied by heart rot (7)	% merch. vol. destroyed by fire (8)	% merch. vol. occupied by sap stain (9)	% merch. vol. occupied by sap rot (10)	% merch. vol. actually deteriorated* (11)	Curved percentage of merch. vol. deteriorated (12)
Jack pine	2	204	11.3	5178.5	3192.8	13.4	1.9	18.2	2.5	22.6	22.0
Jack pine	3	174	11.6	4304.8	2758.0	15.0	1.4	22.5	6.3	31.8	31.1
Jack pine	4	122	12.0	3383.3	2261.3	17.1	0.3	10.9	18.6	33.5	35.5
Jack pine	5	168	11.9	4132.1	2557.6	23.9	0.8	1.5	34.6	47.4	46.7
Red pine	2	96	14.1	4122.4	3372.2	0.4	0.1	46.5	1.5	48.1	38.5
Red pine	3	82	13.3	2759.2	2144.0	1.0	0.1	35.5	5.6	41.8	52.0
Red pine	4	131	14.1	4681.1	3824.2	1.1	—	20.0	36.5	57.5	57.5
Red pine	5	153	13.5	5062.5	4153.5	1.6	—	6.2	51.1	58.6	58.9
White pine	2	26	14.9	1115.1	800.5	8.9	—	27.5	0.4	27.9	27.9
White pine	3	34	13.3	1037.3	752.1	7.0	0.7	25.0	7.4	31.2	35.8
White pine	4	48	14.5	1941.2	1519.1	17.0	—	14.2	25.6	47.9	43.9
White pine	5	48	14.4	1677.8	1305.7	21.3	—	1.7	39.7	53.8	54.3

*Including heart rot increase.

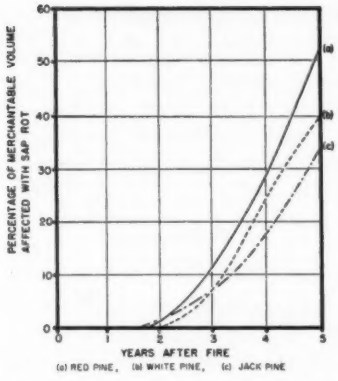

FIG. 20. The increase in the percentage of the merchantable volume of fire-killed jack, red, and white pine containing sap rot during the 5-year period following the fire.

Discussion

The unexpected but striking difference in the rate of increase in volume of heart rot in fire-killed pine compared with that in living trees suggests that this form of deterioration may be a major factor in any study of the deterioration of dead trees. A similar, but less pronounced, relationship was observed in an investigation of the rate of deterioration of balsam fir trees killed as a result of spruce budworm epidemics (2). It may be that balsam with extensive heart rot are more susceptible to the detrimental effects of spruce budworm defoliation, and hence succumbed earlier than sound trees in the same stands. The difference in the rate of increase in volume of heart rot in living and dead trees could be more accurately ascertained in future deterioration studies. This would involve during each year of investigation the felling and examination of several living trees of the same age class close to the dead trees under examination.

Obviously, it is essential that an appraisal of both fungal and insect damage be made when determining the feasibility of carrying out profitable salvage operations under existing economic conditions. Kimmey and Furniss (7) state, "Insects . . . characteristically cause only partial deterioration, as the affected wood is potentially usable as low-grade lumber unless otherwise degraded." Furthermore, significant insect activity in killed timber usually terminates within 2 years, so that although the resultant defects, principally borer tunnels, remain, further deterioration is almost entirely pathological. Nevertheless, the lowering of grade, and hence of value, caused by insect damage and sap stain appears to be of greater economic significance than the reduction in yield of merchantable timber per acre because of wood consumed by fire and sap rot for the 2 or 3 years immediately following the fire. When more than 2 years have elapsed, reductions in yield of considerable magnitude due to deterioration as a direct or indirect result of the fire occur in jack pine and red pine stands.

Acknowledgments

During the course of this study assistance was received from the following: Dr. L. T. White, Forest Pathology Laboratory, Maple; Dr. M. K. Nobles, Botany and Plant Pathology Laboratory, Ottawa; the Ontario Department of Lands and Forests; the Forest Insect Laboratory, Sault Ste. Marie; and the R. F. Tétreault and Kormak Lumber Companies. The help given is gratefully acknowledged.

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EUROPHIUM, A NEW GENUS OF THE ASCOMYCETES WITH A LEPTOGRAPHIUM IMPERFECT STATE[1]

A. K. PARKER[2]

Abstract

The perfect state of a species of *Leptographium* Lag. & Melin has been placed in the new genus *Europhium* of the Eurotiaceae and described as *Europhium trinacriforme* sp. nov. Perithecia were found on the trunks of dead or dying *Pinus monticola* Dougl. and a method was developed for their production in culture.

Introduction

In 1942 Ehrlich and Baker (3) reported the isolation of a species of *Scopularia* Preuss emend. Goid. from dying white pine in Idaho. The same fungus was believed to have been isolated by Gill and Andrews (6) in the United States in 1949 from lesions on white pine affected by pole blight and by Parker *et al.* (14) in Canada. In a later paper Gill *et al.* (7) referred to this fungus as a species of *Leptographium* Lag. & Melin. Subsequent investigation of the nomenclature of *Scopularia*, *Leptographium*, and *Hantzschia* Awd., by Shaw and Hubert (18), resulted in the recommendation that the generic name *Leptographium* be used for the fungus. The fungus has been the organism most frequently isolated from lesions on pole blight trees in the Coastal and Interior[3] regions of British Columbia (15, 16) and inoculation studies have shown it to be capable of producing lesions on healthy pine similar in several respects to those found on pole blight trees (7, 10, 16). Studies on the nature of the association of the fungus with pole blight lesions are under investigation. The present paper describes the perfect state of the fungus and a method of producing it in culture.

Methods

Single-ascospore cultures were obtained by first transferring a mature perithecium through three individual drops of sterile water to free it of conidia. The perithecium was then transferred to a sterile cover slip on the surface of a potato-dextrose agar plate and crushed. A portion of the spore mass was picked up on the point of a sterile dissecting needle and pushed back and forth across the surface of the medium. When germination began, single spores were located under a microscope and if separated sufficiently from one another, each was removed with a dissecting needle and placed on an agar slant.

Measurements were made of 100 ascospores and 100 conidia stained with cotton blue in lactophenol. Measurements of 50 asci were obtained from smear mounts and microtome sections of immature perithecia produced in

[1]Manuscript received November 5, 1956.
Contribution No. 328 from the Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada. Based on part of a thesis presented December, 1955, to the Faculty of Graduate Studies, University of British Columbia, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.
[2]Forest Biology Laboratory, Victoria, B.C.
[3]The term "Interior" is used to refer to all areas of British Columbia east of the Coast Range.

culture. Smear mounts were stained with cotton blue in lactophenol and microtome sections were stained with either carmine or cotton blue. All microtome sections were made from perithecia embedded in paraffin.

The Perfect State

Perithecia in Culture

In the search for the perfect state of *Leptographium* sp. single spores from a number of isolates were paired in all combinations on a variety of media. Water agar, malt agar, potato-dextrose agar, cornmeal agar, and agar containing extracts from white pine were tried without success. Another method tried was the one used by Shafer and Liming (17) for *Ceratostomella ulmi* types, modified by substituting white pine for elm wood sections. Several perithecia without special openings (cleistothecia) were noted on the filter paper beneath the wood section in one of the plates containing the white pine. Attempts to replicate this result failed, but from work on other modifications of this medium a method was developed which resulted in consistent production of perithecia with compatible isolates.

The method adopted for the production of perithecia in culture was as follows. Sections of wood approximately $1 \times 3 \times \frac{1}{4}$ in. were cut from the outer sapwood face of the main stems of white pine after the bark had been discarded. The sections were cut to give a surface of approximately 1×3 in. containing the cambial layer or outer face of the sapwood. The sections were dried for 8 to 12 hours at 70 to 90° C. Each section was then placed on a filter paper in a 9 cm. culture plate with the outer face of the sapwood upwards. Approximately 15 ml. of distilled water was added to each plate before it was autoclaved for 20 minutes at 15 lb. pressure. The purpose of the filter paper was to reduce the free flow of water when the plates were handled. When the plates were cool, isolates were paired by placing a small square of potato-dextrose agar culture from each on the upper surface of the wood section approximately 1 in. apart. Inoculated plates were stored at a room temperature varying from 15 to 25° C. Under these conditions mature perithecia appeared in 3 to 5 weeks on the upper surface of the wood sections where compatible matings had been made. The number of perithecia developing on this medium under these conditions varied from 10 to 50 per wood section. Vegetative growth of the fungus was sparse.

Perithecia on Wood Samples Collected in the Field

Numerous blue-stained wood samples were collected during July, August, and September from white pine slash in stands affected with pole blight. Isolations were made from the wood and from perithecia which occurred on the wood. Perithecia which appeared identical with those obtained in culture were found on some of the samples supporting growth of *Leptographium* sp. These samples originated in stands near Bear Creek on the Coast and near Silverton and West Arrow Park in the Interior. Perithecia were found on the outer face of the sapwood beneath loose and decaying bark.

Several different blue-stain organisms, some having an associated perithecial state, were frequently associated with the perithecia of the *Leptographium* fungus. Although ascospore isolations were made from perithecia of all types occurring on the samples, only the cultures from perithecia resembling those obtained in culture gave growth similar to the *Leptographium* fungus.

The connection between the perithecia and the *Leptographium* fungus was established through observation of the growth arising from single ascospores and single conidia, through interfertility studies with single-ascospore and single-conidial cultures, and through comparison of the results of inoculation experiments made with cultures from both sources.

Description of the Fungus

Because the ascocarp does not have a special opening and the asci are evanescent, this fungus belongs to the Eurotiaceae. However, a relationship to the Ophiostomataceae is indicated because the wall of the ascocarp is black, leathery to brittle, and the ascospores are embedded in a gelatinous matrix on release from the evanescent asci. As the fungus appears to be transitional between members of these families, a new genus, *Europhium*, is proposed; and because the ascospores are enclosed by an envelope resembling a three-pronged or three-cornered hat, the name *Europhium trinacriforme* is proposed.

Europhium gen. nov.

Ascocarpium atrum, orificio speciali vacans, inaequaliter in maturitate erumpens; ascosporae ex ascis evanescentibus in matrice gelatinosa siteae.

Ascocarp black, without special opening, rupturing irregularly at maturity; ascospores in gelatinous matrix on release from evanescent asci.

Europhium ***trinacriforme*** sp. nov.

Mycelium atrum, saeptatum, ramosum, 6.1–9.4 μ diam.; perithecia singula vel aggregata, orificio speciali vacantia, sphaerica, atra, 260 (225–345) μ diam.; paries perithecialis 24–29 μ crassus, pariete exteriore 4–6 cellulia, interiore 1–2 cellulis crassitudine, humectate coriaceus, siccitate fragilis; asci globosi vel subglobosi, 5–9 μ diametro maximo, evanescentes, octosporei; ascosporae hyalinae, unicellulares, trinacriformes, laterali aspectu cucullatae, 4.8 (3.6–5.4) $\times$ 2.2 (1.8–3.1) μ, matrici albidae et gelatinosae, sed in aqua haudquaquam dissolventi, impositae, madore per ruptionem in pariete peritheciali inaequalem, ex qua cirrhi lente ac paulatim protruduntur, expulsae; status conidialis generis *Leptographii*; conidiophora substrata saeptata, 70–600 $\times$ 6.1–9.4 μ, directe e substrato vel mycelio aerio excrescentia, universe singula vel occasione oblata 2–4 aggregata; conidia in ramis apicum conidiophororum hyalinis penicilliformibus producta, cylindracea usque ellipsoideo-ovoidea, hyalina, unicellularia, 5.5 (4.0–7.4) $\times$ 2.5 (1.6–3.8) μ, in massa mucilaginis albidae sita; fungus heterothallicus.

Habitat.: in ligno et cortice arboris moribundi vel emortuae speciei *Pinus monticola* Dougl.

Vegetative mycelium (Figs. 5, 6) black, septate, branched, 6.1–9.4 μ in diameter; perithecia (Fig. 1) occur singly or in groups, without special opening, spherical, black, 260 (225–345) μ in diameter; perithecial wall 24–29 μ, with outer wall 4–6 cells and inner wall 1–2 cells thick, leathery when moist, brittle when dry; asci (Figs. 2, 3) globose to subglobose, 5–9 μ in greatest diameter, evanescent, eight-spored; ascospores (Fig. 4) hyaline, unicellular with three-pronged envelope similar to a three-cornered hat but appearing cucullate in side view, 4.8 (3.6–5.4) $\times$ 2.2 (1.8–3.1) μ including envelope, embedded in white gelatinous matrix that does not disintegrate in water, discharged in the form of a tendril through an irregular rupture in the perithecial wall; conidial state of the *Leptographium* type; conidiophores (Figs. 7, 8) dark, septate, 70–600 $\times$ 6.1–9.4 μ, arising directly from substratum or aerial mycelium, generally occur singly but occasionally in groups of 2 to 4; conidia (Fig. 9) produced on hyaline penicillate branches at the apex of conidiophores, cylindrical to ellipsoid-ovoid, hyaline, unicellular, 5.5 (4.0–7.4) $\times$ 2.5 (1.6–3.8) μ, in white mucilaginous masses at apex of conidiophore; fungus heterothallic.

Habitat: dead or dying sapwood and bark of *Pinus monticola* Dougl.

Type: DAOM 41301, DAVFP[4] 9912. *Pinus monticola* Dougl. Silverton, British Columbia. August 17, 1952.

Specimens examined: CANADA: British Columbia. Silverton, DAOM 41307, DAVFP 9916; DAOM 41306, DAVFP 9917; DAOM 41305, DAVFP 9918. West Arrow Park, DAOM 41308, DAVFP 9915. Duncan, DAOM 41300, DAVFP 9913. Bear Creek, DAOM 41309, DAVFP 9914.

Perithecia in culture: DAOM 41304, DAVFP 9919; DAOM 41303, DAVFP 9920; DAOM 41302, DAVFP 9921; DAOM 41310, DAVFP 9922.

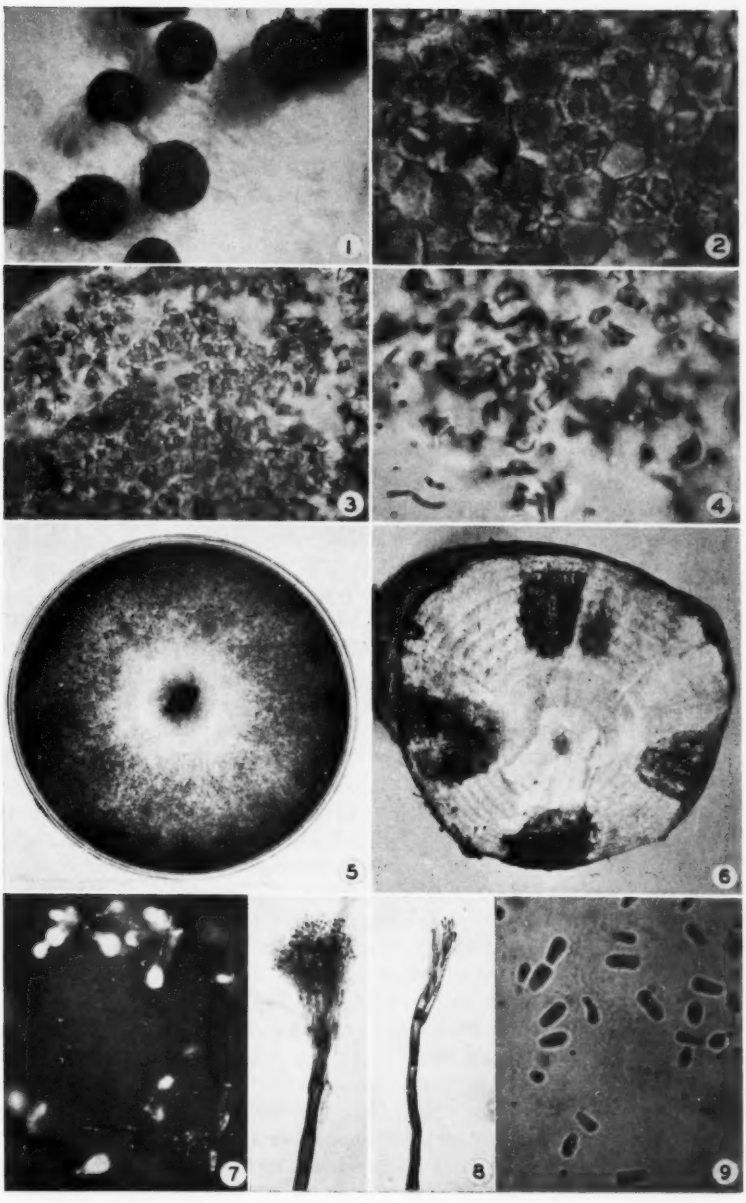
On 3.9% Difco potato-dextrose agar ascospores germinated in 20 to 48 hours and conidia in 16 to 36 hours. The mycelium was white at first but changed to black after 3 to 4 days. The fungus grew approximately 5 cm. in 10 days at 27° C. Conidiophores appeared in 3 to 4 days and coincided with the change in color of the mycelial mat. Conidiophores were extremely variable in length and most of them arose from the aerial mycelium rather than submerged mycelium. Conidium production was prolific and the mucous droplets at the apex of the conidiophores containing the conidia gave a speckled appearance to the cultures. The mucous droplets were white at first but turned a deep yellow to orange color in 5 to 6 days. The droplets remained white, however, when the fungus was grown on 2% malt-extract agar.

[4]Herbarium of the Department of Agriculture, Forest Biology Laboratory, Victoria, B.C., Canada.

FIGS. 1–9. *Europhium trinacriforme.*

FIG. 1. Perithecia, $\times$ 38. 2. Asci with ascospores, $\times$ 1230. 3. Ascospores released from evanescent asci in the perithecial cavity, $\times$ 600. 4. Ascospores with three cornered envelope, appearing cucullate in side view, $\times$ 960. 5. Colony on malt agar after 16 days. 6. Mycelium growing out of the end of a white pine log. 7. Conidiophores with mucous heads containing conidia. 8. Conidiophores, $\times$ 240. 9. Conidia, $\times$ 850.

PLATE I



Parker—Can. J. Botany

Examination of whole perithecia in field collections and in culture did not reveal any consistent pattern in the location of wall ruptures through which the tendrils containing ascospores emerged. Rupturing occurred as jagged slits at the side, top, or bottom of perithecia. Microtome sections through immature and nearly mature perithecia did not reveal any sign of thinning or cracking in the wall. It is assumed that rupture of the wall occurs because of pressure exerted by the spore mass. Asci were found only in very young perithecia. The ascospores were released from asci very soon after being differentiated.

Discussion

Goidànich (8) established the genus *Grosmannia* to accommodate those members of the imperfect genus *Leptographium* Lag. & Melin for which the perfect state had been found. On this basis the fungus described in this paper should be placed in *Grosmannia*. However, Goidànich described the genus as one whose members are ostiolate and beaked and he expected that new members would ordinarily be placed in the genus *Ceratocystis* Ell. & Hals. (Ophiostoma H. & P. Syd.) were it not for the existence of *Grosmannia*. The discovery of *Europhium trinacriforme*, a cleistothecial fungus with a *Leptographium* imperfect state, adds weight to the arguments against placing a strong emphasis on the imperfect states when determining relationships at the present time. Siemaszko (19) and von Arx (1) have objected to *Grosmannia* on the basis that a fungus may have more than one imperfect state and cited *Ceratocystis ips* (Rumb.) C. Moreau as an example. *Ceratocystis galeiformis* Bakshi with *Leptographium*, *Graphium*, and *Cephalosporium* imperfect states may also be cited (2), although future taxonomic studies may show these states to be very closely related.

Europhium trinacriforme belongs to the Plectascales and is here placed in the Eurotiaceae because of its closed perithecia and evanescent asci. A relationship to the Ophiostomataceae is indicated, however, because the ascospores are embedded in a sticky matrix and released from the perithecia in the form of a tendril. Nannfeldt (13), followed by Miller (12) and Gäumann (5), transferred the Ophiostomataceae from the Sphaeriales to the Plectascales on the basis of similarity in centrum characters. They represent an exception to the group, however, as they are ostiolate. The discovery of *Europhium trinacriforme* is additional evidence for the validity of this transfer.

Goidànich (8) proposed a phylogenetic scale within the Plectascales in which the genus *Cephalotheca* Fck., by virtue of the imperfect state and black carbonaceous perithecial walls, was considered to bridge the gap between members of *Eurotium* Link ex Fr. (*Aspergillus*) of the Eurotiaceae and *Microascus* Zukal of the Ophiostomataceae. Nannfeldt (13) and Emmons & Dodge (4) considered that the transition was provided by *Thielavia* Zopf. *Allescheria* Sacc. & Syd. with evanescent asci, closed perithecia, and carbonaceous walls is apparently closely related to *Cephalotheca* and *Thielavia*. *Europhium trinacriforme* would appear to bridge the gap from *Cephalotheca*, *Thielavia*, and *Allescheria*, which have closed perithecia with ascospores

remaining as a powdery mass on release from evanescent asci, and *Microascus*, whose members have ostiolate perithecia with ascospores embedded in a gelatinous matrix on release from evanescent asci. The possibility of another, and perhaps more logical phylogenetic scale, from such genera as *Cephalotheca*, *Allescheria*, and *Thielavia*, through *Europhium* directly to *Ceratocystis* (*Ophiostoma*) is suggested by Grosmann's description of *C. penicillata* (Grosm.) G. Moreau (9). Grosmann describes the beak of *C. penicillata* as being closed until maturity. The apex then dissolves to the extent that pressure created by the enclosed spores is sufficient to tear an opening and allow escape of the spores in the form of a tendril. This process is in contrast to that in those species of the group which have perithecia that are initially closed and at maturity have ostiolar hairs at the apex of the beak. The process which takes place in *C. penicillata* is considered by Nannfeldt (11) to be analogous to the bursting of the wall in the lower Plectascales. Except for the presence of the beak there is little to distinguish the perithecia of *Europhium* from those of *Ceratocystis penicillata*.

Acknowledgments

The author wishes to acknowledge the encouragement and advice received during this study from the late Dr. D. C. Buckland and Dr. F. Dickson of the Department of Biology and Botany, University of British Columbia, Vancouver. He is also grateful to Dr. J. H. Miller, University of Georgia, Athens, for his opinion and helpful suggestions on the classification of the fungus described in this paper; to Dr. J. W. Groves, Department of Agriculture, Ottawa, and Dr. C. G. Shaw, State College of Washington, Pullman, for their helpful criticism of the manuscript; and to Dr. V. Krajina, University of British Columbia, for the Latin diagnosis.

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CYTOGENETIC STUDY ON A TETRAPLOID PLANT AT THE DIPLOID CHROMOSOME LEVEL[1]

KARLIS LESINS

Abstract

It was ascertained that the species from which Grimm alfalfa presumably originated, viz.; *Medicago sativa* and *M. falcata*, may freely exchange genetic material without evidence of a sterility barrier. Consequently a polyhaploid obtained of Grimm may be considered as comparable to natural diploids.

The polyhaploid showed some impaired morphological and physiological characters and pronounced meiotic abnormalities. The single genome ($x = 8$) of the polyhaploid was found to be almost non-functional. Inheritance of purple color indicated a potential autotetraploid mode of transmission of characters.

The polyhaploid was raised back to the tetraploid chromosome level by chromosome doubling and compared to its original and to Grimm. The somatically chromosome-doubled counterpart was found to be a close replica of the original polyhaploid. An assumption that an accumulation of detrimental recessive genes may remain latent at the tetraploid level, and may be expressed upon later reduction to half of the genic variability as in the polyhaploid and its somatically chromosome-doubled derivative, is advanced to explain the observed phenomena.

Phylogenetic, breeding, and culture-historical aspects of the results are discussed.

Introduction

Cytological studies by Ledingham (10) and Julén (9) have shown that cultivated alfalfa, a tetraploid ($2n=32$), is very likely autotetraploid in origin. A haploid plant, $2n=16$, originating as one of three seedlings from a polyembryonic seed of the variety Grimm, was discovered by the author several years ago (11). The plant, subsequently called "polyhaploid" because of the polyploid level of its progenitor, provided a rare opportunity for the study of the evolutionary changes that have occurred in the species during its existence at the tetraploid level and as a cultivated crop, since there are available for study wild-growing, purple- and yellow-flowered strains of diploid, $2n=16$, alfalfa.

In the course of these investigations the first question to be considered was whether the polyhaploid may be regarded as equivalent to natural diploids, that is, whether its parental variety, Grimm, is autotetraploid in origin and may be assumed to be derived from natural diploids. In such a study the variety Grimm itself presents certain difficulties.

The commonly accepted version is that Grimm and other variegated varieties are *Medicago sativa* L. with some introgression of *Medicago falcata* L. If this is correct the question arises whether *M. sativa* and *M. falcata* may freely exchange genetic material without sterility barriers. According to Stebbins (19, 20) these latter characteristics are decisive on the point of whether certain derivatives at higher ploidy level may, or may not, be considered as autopolyploid in origin.

[1]Manuscript received February 16, 1956, and as revised, June 25, 1956.
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If the autotetraploid origin of Grimm was established the polyhaploid could be compared with putative parent diploids as to morphological, physiological, and cytological traits. The genetic makeup of the polyhaploid could be analyzed in inheritance studies. Outcross derivatives would give useful information on trends having taken place in Grimm.

Finally, by raising the polyhaploid back to the tetraploid level, and comparing it to Grimm and to the original polyhaploid, it would be possible to determine what features, if any, may be ascribed to the ploidy level itself.

Materials and Methods

The polyhaploid plant was discovered by checking chromosome numbers of seedlings derived from polyembryonic seeds. Out of 120 polyembryonic seeds, so far examined, only one polyhaploid was obtained. Greenshields (6) did not find any in 55 polyembryonic seeds tested. Thus, polyhaploids occur rarely in polyembryonic seeds of alfalfa.

The polyhaploid, though completely self- and male-sterile, had some female fertility. The first crosses to *M. falcata* produced two descendants. A good deal of attention has been given to these plants, which will hereafter be denoted as 240–1 and 240–2. Later 11 more offspring were obtained, all of which showed true hybridity by the green flower color invariably present in *M. sativa* × *M. falcata* crosses.

Diploid *M. sativa* material was represented by strains from east Caucasus (U.S.S.R.), Iran, Georgia (U.S.S.R.), and Armenia (U.S.S.R.). The *M. falcata* types were from south Germany, northwest Caucasus (U.S.S.R.), and from central Siberia (U.S.S.R.). The above forms and their intercross products were used for comparison and in inheritance studies.

An intermediate type, *M. hemicycla* Grossh. (Wisconsin accession No. 16693) was from Transcaucasus. Plants of variety Grimm originated from certified seed.

Techniques for cytological studies, for making cross-pollinations in all critical crosses, for chromosome doubling, and for rooting cuttings were those described previously by the present author (12, 13).

Results

1. Studies of Crosses of *M. sativa* × *M. falcata* at the Diploid and Tetraploid Level

These studies were made primarily to establish the relationship between *M. sativa* and *M. falcata* as related to the origin of variegated alfalfa. The main distinguishing characters of the species are: coiled pods and purple flowers for *M. sativa*, and straight to sickle pods and yellow flowers for *M. falcata*.

(a) Flower Color and Pod Shape

Data on inheritance of flower color in crosses of *M. sativa* × *M. falcata* at the diploid and tetraploid level are presented in Table I; data on inheritance of degree of pod coiling are given in Table II.

TABLE I

GENETIC RELATIONSHIP OF *M. sativa* AND *M. falcata* AS INDICATED BY SEGREGATION OF PURPLE FLOWER COLOR IN THEIR CROSSES AT THE DIPLOID AND TETRAPLOID LEVEL

Provenance of strains intercrossed		At diploid level				At artificial tetraploid level			
		Generation, number or desig. of families	No. of plants with flower color		Ratio and *P* values (in parentheses)	Generation, number or desig. of families	No. of plants with flower color		Ratio and *P* values (in parentheses)
M. sativa	*M. falcata*		Purple	Non-purple (near-white in parentheses)			Purple	Non-purple	
East Caucasus	N.W. Caucasus	F_2, 6 fam.	118	33 (3)	3:1 (0.5–0.3)	F_2, 2 fam.	165	2	35:1 (0.3–0.2)
		B_1, 4 fam.	206	224	1:1 (0.5–0.3)	B_1, 1 fam.	11	1	5:1 (0.5–0.3)
Georgia	N.W. Caucasus					F_2, 1 fam.	74	3	35:1 (0.7–0.5)
Georgia	S. Germany	F_2, Fam. *a*	20	10 (0)	3:1 (0.3–0.2)	F_2, 2 fam.	1058	23	35:1 (0.2–0.1)
		F_2, Fam. *b*	100	49 (3)	3:1 (0.05–0.01)				
		B_1, 1 fam.	23	24	1:1 (0.9–0.7)				
Iran	C. Siberia	F_2, Fam. *c*	144	47 (4)	3:1 (0.9–0.7)				
		F_2, Fam. *d*	433	180 (6)	3:1 (0.05–0.01)				
		B_1, 2 fam.	97	82	1:1 (0.3–0.2)				
Armenia	C. Siberia	F_2, 1 fam.	115	29 (3)	3:1 (0.2–0.1)				
		B_1, 1 fam.	42	53	1:1 (0.3–0.2)				
	Total	F_2, 11 fam.	930	348 (19)	3:1 (0.1–0.05)	F_2, 5 fam.	1297	29	35:1 (0.2–0.1)
		B_1, 8 fam.	368	383	1:1 (0.7–0.5)	B_1, 1 fam.	11	1	5:1 (0.5–0.3)

TABLE II

DEGREE OF POD COILING, EXPRESSED IN EIGHTHS OF A CIRCLE, IN F_2 PROGENIES OF THE DIPLOID CROSS, *M. sativa* × *M. falcata*

Provenance of strains intercrossed		Desig. of families	Frequency of plants in pod coiling classes									
M. sativa	*M. falcata*		0–2 (*falcata*)	3–4	5–6	7–8	9–10	11–12	13–14	15–16	17 and over (*sativa*)	Total
E. Caucasus	N. W. Caucasus	No. 213	0	1	9	12	22	11	4	1	0	60
Georgia	S. Germany	No. 227	0	0	2	5	6	6	3	3	0	25
Georgia	S. Germany	No. 230	1	8	12	27	39	14	6	6	0	113
		Total	1	9	23	44	67	31	13	10	0	198

In flower-color studies, bulked family ratios did not deviate significantly from the expected on the basis of purple flower color being governed by one main gene. In families denoted "*b*" and "*d*" the *P* values were between 0.05 and 0.01, that is, on the verge of significance. This disparity may be attributed to differential genotypic mortality as often encountered on the selfing of cross-pollinated plants. Thus, in families "*b*" and "*d*" there was 28 and 27% seedling mortality, respectively. The possibility of complementary factors for purple flowers, giving a 9:7 ratio of purples to non-purples, should not be ruled out, though in the present study the fit to 9:7 is much poorer than that to a 3:1 ratio, even for the deviating families. It should be pointed out that Twamley (22) in crosses of natural diploids of *M. sativa* × *M. falcata* found purple flower color to be governed by one factor as a rule, but in certain crosses the action of two complementary factors was indicated.

The occurrence of a small proportion of near-white (light cream) flower types in diploid F_2 families suggests that together with one factor for purple, there are segregating two factors for yellow color. The ratio expected on this basis, 63 colored: 1 non-colored, was obtained in separate families, as well as from the total results. The two factors for yellowness, however, should be considered as major ones, because selfing of one of the near-white plants gave some "strong-cream" flower types. Investigations on this question are now in progress.

These results from interspecific crosses at the diploid level do not preclude the possibility of non-random pairing if strains were raised artificially to the tetraploid level. At the tetraploid level, characters determined by several genes do not provide a useful means for segregation studies because of the large population needed. Purple flower coloration, however, being monofactorially inherited at the diploid level, could be used, and in Table I it may be seen that its segregation at the tetraploid level has followed, very probably, an autotetraploid pattern. In the F_2 the chromosome segregation ratio, 35:1, was assumed and χ^2 tests showed good fits to actual data. The chromatid segregation ratio, 21:1, may not be wholly ruled out because of limited populations studied.

Data on pod shape were classified using intervals of 1/8 coil, with *M. sativa* having usually more than 2 coils, *M. falcata* 0 to $\frac{1}{4}$, and the F_1's at most $1\frac{1}{4}$ coils per pod. On F_2 plants, several racemes were hand-intercrossed in addition to those that were open-pollinated. From resulting pods the most-coiled one was taken for coilage measurement; thus was obtained a value for the highest coilage potential possessed by a particular plant. From the data in Table II it is obvious that inheritance of pod shape is governed by more than one gene, and that the genes are cumulative in their effect.

It may be concluded, therefore, that there was free exchange of genetic material between *M. sativa* and M. *falcata* relative to characters considered as distinguishing the species.

TABLE III

FERTILITY IN CROSSES WITHIN AND BETWEEN STRAINS OF DIPLOID *M. falcata* AND *M. sativa* AND BETWEEN THEIR F_1 HYBRIDS

Strains intercrossed	No. flowers crossed	Per 100 flowers	
		No. pods set	No. seeds set
M. sativa (Iran) × *sativa* (E. Caucasus)	201	58	144
M. falcata (N.W. Caucasus) × *falcata* (C. Siberia)	200	78	314
M. sativa (Iran) × *M. falcata* (C. Siberia)	206	71	243
F_1 [*M. falcata* (C. Siberia) × *sativa* (Iran)] × F_1 [*M. falcata* (N.W. Caucasus) × *sativa* (E. Caucasus)]	195	87	341

(b) Interfertility Studies

Data on fertility of crosses between diploid *M. sativa* and *M. falcata* are presented in Table III.

This point is important in establishing phylogenetic relationships. As can be seen, there was no reduction in fertility in crosses of F_1 hybrids as compared to *intra*specific crosses in which *M. sativa* or *M. falcata* of different provenances were involved.

Full fertility in crosses of *M. sativa* and *M. falcata* was reported also by Bolton and Greenshields (2), and Oldemeyer and Brink (15) have shown that fertility in offspring was not reduced if the genomes of diploid *M. falcata* were joined with genomes of cultivated alfalfa.

It is significant, too, that there has been no observation of reduction in vigor of plants attributable to intercrossing of *M. sativa* × *M. falcata*.

Hence it may be concluded that the variety Grimm, even though presumably having both *M. sativa* and *M. falcata* elements in its parentage, should be considered as an autotetraploid because the putative parents may freely exchange genetic material, and there are no sterility barriers. This is very likely applicable to any other cultivated variegated-flowered alfalfa. Moreover, it follows that the polyhaploid can be validly compared to diploid *M. sativa*, *M. falcata*, or their hybrids.

2. COMPARISON OF THE POLYHAPLOID WITH ITS OFFSPRING, NATURAL DIPLOIDS, AND THEIR F_1'S

While in the foregoing it was concluded that the polyhaploid could validly be compared to diploid *M. sativa*, *M. falcata*, and their hybrids, the possibility has been proposed that variegated alfalfas may have arisen from a separate wild growing species, *M. media* Pers. (*M. varia* Mart.). This is a tetraploid (and, therefore, should be phylogenetically younger) and originated from some diploid itself. The only diploid form known which covers a flower color range from purple to yellow is *M. hemicycla*. It was therefore also compared with the polyhaploid.

(a) Morphology

Erect growth habit characteristic of cultivated alfalfa was the most conspicuous character distinguishing the polyhaploid from natural diploids, from F_1's of *M. sativa* × *M. falcata*, and from offspring derived from the polyhaploid crossed to *M. falcata*. Its stems appeared softer, but thicker and more brittle than those of natural diploids and their hybrids. Stems of *M. hemicycla* characterized as "wiry" were especially deviating.

The flower color of the polyhaploid was purple with a bluish tinge. Such color was not found in diploid *M. sativa* nor in F_1's of the cross *M. sativa* × *M. falcata*, which are greenish flowered. In subsequent generations of the latter cross such coloration was found. In *M. hemicycla* flower color similar to that of the polyhaploid was not noted; the population studied, however, comprised only about 30 plants.

The polyhaploid exhibited an abnormality of some flowers, especially in the apices of racemes. Affected flowers had sometimes irregularly shaped petals with open keels exposing the staminal columns, the latter often being without tension, which is characteristic for normal flowers. No other plants showed this trait.

An important abnormality of the polyhaploid was its non-dehiscent anthers containing only a few stainable pollen grains. Results on pollen quality as well as on meiosis are given in Table IV.

The 15% stainable pollen of the polyhaploid, however, should not be considered as viable grains because their diameters ranged from 22 to 65 μ, which extends below the diameters for normal diploid (35 μ) and above that for artificial octoploid (47 μ) pollen. This indicates extreme variation in amount and unbalance of genetic material included in different pollen grains, resulting in inviability. The offspring of the polyhaploid, 240–1, produced more normal pollen, as may be seen by referring to Figs. 3 and 4. The data show that pollen quality was highest in F_1 plants of *M. sativa* × *M. falcata* and in *M. hemicycla*. In comparison with other diploids, the polyhaploid had the lowest number of pollen grains per anther as indicated by the average of two to three replicate counts for anthers from the non-fused filaments.

In connection with the genic makeup of the polyhaploid to be discussed later, morphological abnormalities of plant 240–2, an offspring of the cross polyhaploid × *M. falcata*, are of interest. Its stems and leaves were much thinner and smaller than those of the polyhaploid or any plant observed. Moreover it apparently had two different growth phases: one "vegetative" with very short stem internodes causing plant dwarfing; the other "reproductive" in which the internodes elongated and the plant reached normal height. Branches representing these phases in comparison to those of a sister plant, 240–1, are illustrated in Fig. 1. Flowers of 240–2, while almost as long as normals, had narrow petals having about one-third the weight of normal petals.

TABLE IV

DATA ON CHROMOSOME BEHAVIOR AT MEIOTIC PHASES AND ON POLLEN QUALITY OF THE POLYHAPLOID AND OF OTHER COMPARABLE PLANTS

Plant material	Meiotic cells examined								Pollen	
	Diakinesis		Metaphase I		Telophase I		Telophase II			
	Total no.	Abnorm., %	Total no.	Abnorm., %	Total no.	Abnorm., %	Total no.	Abnorm., %	No. per anther	Stainable, %
Polyhaploid	222	29	62	32	790	9	264	25	257	15
240–1	99	16	37	14	819	6	164	10	491	45
F_1, *M. falcata* (S. Germany) × *sativa* (Georgia)	35	3	24	13	174	1	34	6	433	73
F_1, *M. falcata* (C. Siberia) × *sativa* (Armenia)	44	4	10	—	446	1	48	—	501	76
F_1, *M. falcata* (N.W. Caucasus) × *sativa* (Georgia)	62	11	40	8	209	2	52	4	541	93
M. hemicycla, pl. No. 1	158	8	30	13	141	7	58	7	391	86
M. hemicycla, pl. No. 2	17	6	1	—	163	0	40	3	370	79

(b) Physiology

The polyhaploid was characterized by a general reduction in rate of top and root growth, and by necrotic leaf tips, especially apparent in the greenhouse after changes in growing conditions such as application of fertilizers or an accidental period of drought. F_1's of *M. sativa* × *M. falcata* or offspring of the polyhaploid did not exhibit such symptoms, but the latter plants sometimes shed part of their leaves in response to changes in growth conditions.

None of the $n=8$ pollen of the polyhaploid was functional either in selfing or in crossing with other diploid plants even when pollen was collected from a hundred or more flowers. While, on selfing, the polyhaploid produced a few seeds, none of the resulting plants had the expected chromosome number, $2n=16$. Of 12 seedlings obtained from intercrossing these plants, 11 had 32 and one had 33 chromosomes in the root tips. Obviously only pollen grains and eggs with chromosome numbers close to $n=16$ functioned and the tetraploid level was rapidly restored.

A few of the $n=8$ female gametes of the polyhaploid were functional when crossed with natural diploids and one to eight seeds were produced per 100 flowers crossed. When crossed with tetraploids, a seed set of 86 per 100 flowers was obtained, indicating again that the non-reduced chromosome complement $n=16$ and not the reduced $n=8$ was usually functional.

The offspring plant 240–2, mentioned for its morphological features, had non-functional pollen, but, as a pistillate parent, gave 20 to 30 seeds per 100 flowers, a much better set than that of the polyhaploid. Its environmental requirements for flowering were peculiar. Unlike other plants no flower buds were formed during the winter in the greenhouse, and during the summer in the field flower buds showed pronounced abortive tendencies. Flowering was successful only in the greenhouse in summer, where daylight was supplemented with continuous artificial light.

(c) Cytology

Contrary to expectations no somatic chromosome abnormalities or differences were found between the polyhaploid and its most deviating offspring, 240–2 (Figs. 6 and 7), or between it and normal diploid plants.

Results on meiotic behavior of chromosomes of the polyhaploid, offspring 240–1, three different F_1 plants of *M. falcata* × *M. sativa*, and two plants of *M. hemicycla* are presented in Table IV.

Because of difficulties arising from the smallness, crowding, and stickiness of chromosomes in meiotic stages, only a small number of diakinesis and metaphase I cells could be analyzed.

Cells in late diplotene to metaphase I in which eight chromosomal bodies (assumed to be eight bivalents) were observed were classed as normal. Those with fewer or more than eight were classed as abnormal. Undoubtedly, in cells with more than eight bodies, univalents were present, while in those with fewer than eight (this group about 1/3 of all abnormals) multivalents would be indicated, though some configurations might have been held together by stickiness of the matrix. Fig. 8 shows the normal late diplotene

chromosome associations in the polyhaploid; Figs. 9 and 10 show chromosome association in seven and nine configurations respectively. Fig. 12 shows eight configurations at metaphase, while in Fig. 13 seven configurations, in Fig. 14 probably six configurations and a non-oriented bivalent, and in Fig. 15 four univalents may be seen.

Cells without laggards or chromatin bridges (these latter encountered very seldom) at anaphase and telophase (given under telophase in Table IV) were considered as normal. Abnormal anaphase – telophase I and II cells with one or more laggards are illustrated in Figs. 16 to 19.

In the polyhaploid a few cells were observed in which chromosome condensation at late diplotene was not synchronous. A cell in which some configurations are clearly visible while others are indistinct is illustrated in Fig. 11.

From data in Table IV it is evident that, in the polyhaploid, chromosomal abnormalities at every meiotic stage were much more frequent than in any F_1 hybrid or in *M. hemicycla*. Plant 240–1 was intermediate in this respect.

The comparatively regular meiotic behavior of the F_1 hybrids and *M. hemicycla* indicate that the genomes of cultivated alfalfa have undergone considerable changes. Structural rearrangements such as reciprocal translocations and inversions may have occurred, or single genomes may have accumulated recessive genes, some of which might impair the meiotic processes. The latter appears most likely since about 2/3 of all abnormal metaphase I and diakinesis cells had univalents. This is supported by the fact that a more normal meiosis is restored when the genome of the polyhaploid is combined with that of a natural diploid as in plant 240–1. Many cases of such gene-controlled meiotic abnormalities, especially asynapsis, have been compiled by Gaul (5).

The higher percentage of abnormalities in telophase II compared with telophase I may indicate that some univalent chromosomes were dividing during first anaphase and lagging at second, and/or that specific impairing factors were causing chromosome lagging during the second anaphase.

While the number of normal appearing tetrads observed would indicate a fair amount of good pollen, many pollen grains obviously aborted during their post-tetrad development, possibly as a result of genic unbalance governing their nourishment.

(d) Inheritance Studies

As shown earlier, free exchange of genetic material takes place in crosses between *M. sativa* and *M. falcata*. If the polyhaploid is equivalent to these, it should show the same kind of inheritance.

Purple flower color, being more simply inherited than the other distinctive characters, was selected for inheritance tests. The results are presented in Table V.

All 13 offspring from crosses of the polyhaploid to two different strains of yellow falcatas had purple coloration. Obviously both chromosomes of the polyhaploid had the factor for purple. Hybrids 240–1 and 240–2 (derived from polyhaploid × *M. falcata*) when backcrossed to falcatas or to Ivory

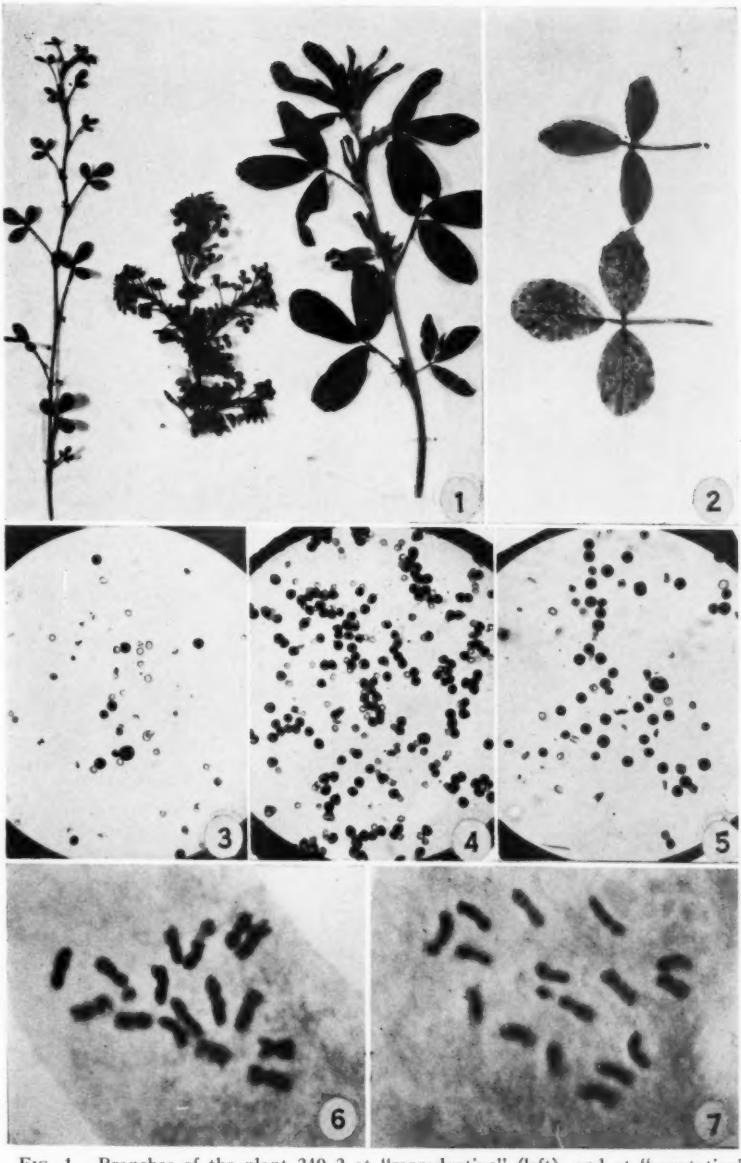
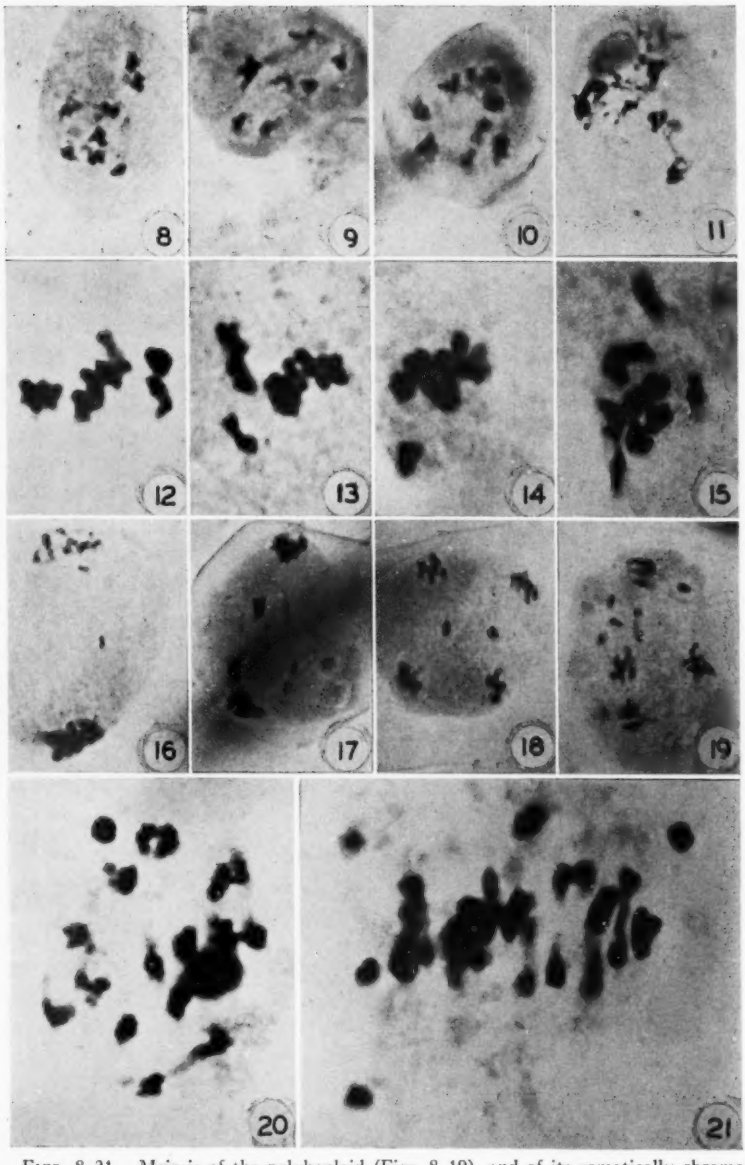


FIG. 1. Branches of the plant 240–2 at "reproductive" (left), and at "vegetative" (middle) phases compared to a branch of its normal sister plant (right). FIG. 2. Old, diseased leaves of the polyhaploid (above), and of its somatically chromosome-doubled counterpart (below). FIGS. 3–5. Pollen grains of the polyhaploid (Fig. 3), of its offspring 240–1 (Fig. 4), and of its somatically chromosome-doubled counterpart (Fig. 5). FIGS. 6, 7. Somatic-chromosome complements of the polyhaploid (Fig. 6) and of its offspring, 240–2 (Fig. 7).

FIGS. 1, 2, 3/4 nat. size; FIGS. 3–5, × 46; FIGS. 6, 7, × 2400.



FIGS. 8–21. Meiosis of the polyhaploid (Figs. 8–19), and of its somatically chromosome-doubled counterpart (Figs. 20, 21). FIGS. 8–11. Late diplotene with eight-, seven-, and nine-chromosome configurations, and with an asynchronous chromosome condensation, respectively. FIGS. 12–15. M I with eight-, seven-, six-, and 10-chromosomal configurations. FIGS. 16, 17. T I with one and several laggards. FIGS. 18, 19. T II (A II) with two and several laggards. FIG. 20. Late diplotene with 15-chromosomal configurations. FIG. 21. Beginning of A I with probably 13 II and 6 I. FIGS. 8–11 and 16–19, × 1000; FIG. 20, × 1700; FIGS. 12–15 and 21, × 2400.

TABLE V

INHERITANCE OF PURPLE FLOWER COLOR IN CROSSES INVOLVING THE POLYHAPLOID AND ITS DESCENDANTS

Cross or self	No. of plants with flower color		Ratio expected and P values (in parentheses)
	Purple	Non-purple	
Polyhaploid × *M. falcata* (S. Germany)	2	0	1:0
Polyhaploid × *M. falcata* (C. Siberia)	11	0	1:0
240–1, self	5	2	3:1 (0.5–0.3)
240–1 × *M. falcata* (C. Siberia)	18	24	1:1 (0.5–0.3)
240–1 × Ivory	24	20	1:1 (0.7–0.5)
Ivory × 240–1	16	20	1:1 (0.7–0.5)
240–2 × *M. falcata* (C. Siberia)	5	13	1:1 (0.1–0.05)

(a non-purple segregate in F_2 from cross *M. sativa* × *M. falcata*), gave ratios of 1:1. This monohybrid ratio is also obtained when natural diploid *M. sativa* × *M. falcata* is backcrossed to *falcata* (Table I). It may be inferred, then, that at the tetraploid level there should be a tetrasomic mode of inheritance, which was actually found for *M. sativa* × *M. falcata* hybrids in this study (Table I), and which was reported for cultivated alfalfa by Stanford (16).

The few offspring plants obtained on selfing 240–1 give some indication of the genic makeup of the polyhaploid itself. The severe unbalance of genic makeup of the polyhaploid is indicated by its self sterility and male sterility, and by the functioning of only a few $n=8$ eggs. Its offspring 240–1, on the other hand, had a better constitution and produced on selfing about one pod per raceme. The pods, however, contained mostly shrivelled non-viable seed and even the plump seed that germinated produced weak seedlings that seldom reached the flowering stage. Thus, from 30 apparently good seeds only seven flowering plants could be obtained.

Plant 240–2 also provided some information on the genetic constitution of the polyhaploid plant. While also self and male sterile, it was more female fertile than the polyhaploid. In the cross to *M. falcata*, it gave 16 normal plants to 9 with the 240–2-like character. Thus a reasonable fit to a 1:1 ratio was obtained.

As to the possible origin of plant 240–2, it may have originated as a dominant mutation at gametogenesis, probably in the egg. From the evolutionary standpoint it may also be postulated that the mutation was present in the polyhaploid, but was covered by a stronger normal-like allele or a linked suppression gene inhibiting its manifestation. Because of strongly abnormal type of growth and because there are no natural conditions permitting reproduction, any 240–2-like mutation in a diploid would not survive. In a tetraploid, however, the mutation would be counteracted by its three normal alleles, perhaps resulting only in weakened vegetative vigor and reproduction. Because the plant is perennial, there are possibilities of a second mutation

neutralizing the first. Such a mutation, favored by natural selection, might become established in the population. It would represent a qualitative change brought about because of the tetraploid condition. Non-appearance of the 240–2 mutation in the other cross of the polyhaploid with a different *M. falcata* strain may be because this mutation only rarely gives viable seedlings if combined with a genome of a natural diploid, or, in the case of a linked suppressor, requires crossing over (occurring rarely) for its recovery.

3. Comparisons between the Polyhaploid, Its Chromosome-doubled Derivative, and Grimm

To determine whether the deviating characters of the polyhaploid were caused solely by ploidy level per se, shoots from rooted cuttings of colchicine treated plants showing conspicuously broader leaves (Fig. 2) and larger flowers were cytologically investigated, and those showing the doubled chromosome number were compared with the polyhaploid and Grimm.

The doubled counterpart had broader leaves, larger flowers, and a more robust general appearance than the polyhaploid. Grimm plants showed these characters to varying degrees. In contrast to Grimm, the chromosome-doubled plants and the original polyhaploid had similar flower abnormalities, viz., distorted petals and open keels occurring sometimes in flowers at the tips of racemes.

The chromosome-doubled and the original plants were strikingly similar physiologically as shown by slow root and shoot development as contrasted to Grimm plants. They were, further, noticeably similar in susceptibility to leaf spot disease (Fig. 2).

Results of pollen quality and meiotic telophases from chromosome-doubled, the original, and Grimm plants are presented in Table VI. Pollen quality was better in chromosome-doubled plants than in the original (Figs. 3 and 5), and there was less variation in pollen diameter (28 to 53 μ) compared to the non-doubled plant (22 to 65 μ). Pollen quantities were similar, indicating that factors controlling production of pollen were fully operative at the two-factor dosage of the polyhaploid. Pollen quantities varied rather widely in different Grimm plants.

Six seeds were obtained from 100 flowers of natural tetraploids pollinated with pollen of the chromosome-doubled plant; likely not all stainable pollen was functional. When this cross was reciprocated, 240 seeds were obtained from 100 flowers, i.e., three times more than before chromosome doubling.

Because of crowding and stickiness, cytological analyses of the chromosome-doubled plant and Grimm plants had to be restricted to anaphases and telophases, and a precise determination of associations at metaphase I was precluded. In the chromosome-doubled plant, 61 late diplotene to early anaphase I cells were examined and the number of configurations appeared to range from 11 to 26. It appeared that pairing was predominantly bivalent with a high incidence of other configurations, especially univalents, as in the original. Figs. 20 and 21 show cells with typical chromosome configurations.

TABLE VI

DATA ON MEIOTIC TELOPHASES AND ON POLLEN QUALITY IN THE SOMATICALLY CHROMOSOME-DOUBLED COUNTERPART OF THE POLYHAPLOID IN COMPARISON TO THE ORIGINAL AND TO SOME GRIMM PLANTS

Plant material	Meiotic cells examined				Pollen	
	Telophase I		Telophase II			
	Total no.	Abnorm., %	Total no.	Abnorm., %	No. per anther	Stainable, %
Polyhaploid, original	790	9	264	25	257	15
Polyhaploid, chromosome-doubled	138	20	94	31	292	30
Grimm, plant No. 1	147	2	31	3	597	96
Grimm, plant No. 2	104	2	17	—	383	81
Grimm, plant No. 3	89	2	21	5	443	41
Grimm, plant No. 4	110	0	30	—	214	78
Grimm, plant No. 5	134	0	47	4	452	96

The main abnormality in the chromosome-doubled plant was, as in the original polyhaploid, the occurrence of lagging chromosomes at anaphase and telophase. If the meiotic abnormalities in the polyhaploid had been caused by non-homology of single chromosomes as in true haploids or in autotetraploids in which structural "diploidization" had occurred, then chromosome doubling should have brought about a general rehabilitation of the meiotic pairing. Actually, the percentage of abnormal telophases I was twice that of the original polyhaploid. Apparently the abnormalities were genic, and by doubling the chromosomes, the effect was at least partially cumulative.

In Grimm plants, only few abnormal cells were found as compared to the number found in the polyhaploid and its tetraploid derivative. This is understandable since in natural plants there are four chromosomes, each able to carry a dominant gene which may neutralize the effect of three recessive harmful alleles. Consequently, it might require an enormous sample of natural plants to discover one similar, genetically, to the chromosomally-doubled derivative of the polyhaploid.

Genic control of pollen production is evident also in Grimm plant No. 3, which produced only 41% good pollen though its chromosomal abnormalities were scarcely higher than in the other Grimm plants. It should be pointed out that the chromosome-doubled derivative had many more abnormalities and still produced twice as much good pollen as its original. The better pollen quality may be explained on the assumption that in the doubled part gametes with two different genomes may be formed and lethals in one genome may be counterbalanced by normal genes in the other.

Discussion

The above reported findings on the polyhaploid is what could be expected realizing that all cross-pollinated plants harbor an immense supply of recessive, harmful genes. As Gustafsson (7) put it: "The populations are stuffed with

lethals in heterozygous condition." It is a commonplace thing to expect and find that when cross-pollinators are selfed all the offspring have decreased vitality because one or other of detrimental genes has been disclosed in a homozygous condition. Only a little consideration is needed to see that at the autotetraploid level under mutation pressure there will be a much higher accumulation of detrimental genes than at the diploid level. On the intercrossing of individuals having half the alleles of a locus of the recessive type, there will be eliminated as fully recessives only one out of 36 at the tetraploid, but one out of four at the diploid, level. Cultivated alfalfa is a plant both naturally cross-pollinated and autotetraploid in origin, hence the constitution and behavior of its polyhaploid. It is because of the light that may be cast on some general problems that the results on the polyhaploid should be discussed.

Firstly there is the problem of the phylogenetic origin of some polyploids. In the last decade a trend of thought has been prevalent ascribing to most of the natural polyploids origin as alloploids, especially as segmental alloploids. The situation has been puzzling mostly because of the impossibility of obtaining proof of the relationship of the possible parental species. Segmental allopolyploids as defined by Stebbins (19, 20) imply the production of sterility when both of the parental genomes are joined at the diploid level. Unfortunately, in most cases the possibility may not be disregarded that species which might have been to some extent intersterile at the diploid level have been parents, but have become extinct or have not yet been discovered. The crucial test of sterility at the diploid level not being available, other characteristics have been used as indicators of alloploid derivation. Among these are sterility of the polyhaploids, irregularities of chromosome pairing at meiosis, and disomic inheritance ratios. In this study it was shown that sterility in the polyhaploid and irregularities in its meiotic processes may have been caused by degradation of the genic complement of the single genome during a long period of maintenance at the tetraploid chromosome level. A strong support for this view is the case of the potato. Ivanovskaja (8) deduced that potato is of alloploid origin because she found sterility in the haploid (polyhaploid) plant. This assumption had later to be corrected because findings by Swaminathan (21) and Nantke (14) indicated that cultivated potato was autopolyploid in origin. In alfalfa there is available a short notice by Stanford and Clement (18) that another polyhaploid has been found and that it is morphologically and cytologically similar to the one reported here.

Such degraded genic complements as discussed above imply that restoration of fertility after chromosome-doubling may not always take place in interspecific hybrids. Application of selected self-fertile lines to interspecific crosses may produce more vital offspring than normally cross-pollinated material.

Secondly, there is the question of disomic and tetrasomic segregation ratios. Their importance for the plant breeder is obvious. Not a small significance

has been attached to it also in solving phylogenetic problems. Before the tetraploid nature of alfalfa was clearly recognized, inheritance data were interpreted on the disomic basis (cf. Atwood and Grun(1)). Later tetrasomic ratios were shown to occur (16, 17, 3) but recently a disomic ratio again has been reported (4). In this study there have been no indications that a deviation from the tetraploid mode of inheritance takes place. Regarding the disomic ratios, if they are ascertained in further studies, it will be of great interest from the evolutionary point of view to see whether diploidization of certain chromosomes has been dictated by the tetraploid level per se or has been established by chance. Decisive information on this will be gained when identical types of mutants from different populations are investigated regarding their mode of inheritance. If diploid and tetraploid ratios are found for the same type of mutant, we may conclude that diploidization occurs by chance, possibly influenced by non-homogeneous accumulation of recessive genes. It is likely that such chance diploidizations are eliminated on intercrossing with normal plants, and a cross-pollinated crop like alfalfa retains on the whole its autotetraploid mode of inheritance.

Thirdly, some thought may be given to the significance of tetraploidy and of cultivation by man to evolution of a crop like alfalfa. On doubling the polyhaploid, no apparent changes in any vital characters were found. This is what may be expected of a plain doubling of chromosome complement. To judge the possibilities of ploidization by this first product would, however, be wrong. As indicated by the case of plant 240–2 there may be preservation of a certain mutation and pressure for another mutation in connection with the first one that may be ascribed to the tetraploid level alone. It scarcely needs mentioning that a cumulative effect of dosage of four instead of only two factors may be of importance for some characters.

Alfalfa seems to be a unique crop in that its history of cultivation is too old to be followed down to its beginning, and too young to warrant the assumption that its wild ancestors have died out or have undergone thorough evolutionary changes, as is likely the case with the main grain crops. Thus, characters of cultivated alfalfa such as erect growth habit, rapid recovery after cutting, sturdy though tender texture of stems, and a very high mass production are likely made possible by tetraploidy and developed by man because they are not encountered to that degree in any wild growing diploid or tetraploid alfalfas.

Lastly, it may be indicated that the line of investigation—comparing polyhaploids of autotetraploids with their phylogenetically older diploid forms—may make a contribution to our understanding of evolution of plant species. Differences that might be found in cross-pollinated species as compared to self-pollinated ones may be very informative. There are a few cultivated crops such as alfalfa and potato whose wild growing diploid ancestors may be traced down, and a good many non-cultivated species with both diploid and tetraploid forms available for investigation.

Acknowledgments

I am indebted to Drs. J. Unrau, L. P. V. Johnson, J. R. Fryer, and G. Walker, University of Alberta, for help in preparation of the manuscript. To my wife, Irma, I am indebted for technical assistance throughout these studies.

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ADDITIONS TO THE PARASITIC FUNGI OF NOVA SCOTIA[1]

D. B. O. SAVILE

Abstract

The following parasitic fungi are newly reported from Nova Scotia: *Peronospora cakiles* Savile sp. nov. (on *Cakile edentula*), *Taphrina tosquinetii* (West.) Magn., *Stigmatea robertiani* Fr., *Pucciniastrum potentillae* Kom., *Puccinia calcitrapae* DC., *Puccinia praegracilis* Arth. var. *cabotiana* Savile var. nov. (on *Hierochloë odorata*, aecia on *Habenaria psycodes*), *Uromyces ervi* West., *Cercospora pontederiae* Ell. & Dearn., *Isariopsis bulbigera* (Fckl.) Savile comb. nov. (= *Ovularia bulbigera* (Fckl.) Sacc.), *Ramularia oxalidis* Farl., *Ramularia pusilla* Unger (= *Ovularia pusilla* (Unger) Sacc.), *Ramularia rubella* (Bon.) Nannf., *Ramularia subsanguinea* (Ell. & Ev.) Savile comb. nov., *Marssonina sennenis* Gonz. Frag., *Septogloeum potentillae* Allescher, and *Septoria levistici* West. The status of the genera *Ovularia* and *Ramularia* is briefly discussed. A few new host records of other fungi are included.

Wehmeyer (12), in his account of the fungi of the Maritime Provinces, summarizes the knowledge of the fungi of Nova Scotia. The late W. P. Fraser made detailed studies of the Erysiphales and Uredinales, especially in Pictou Co., and the fungi attacking cultivated plants have received much attention, but examination of the records of parasites of the native vegetation suggested that selective collecting in certain habitats might be profitable. During a visit to Nova Scotia in August 1956, I accordingly took the opportunity to collect in coniferous woods, bogs and, especially, in the varied littoral habitats. The resulting records of fungi new to the province and a few new host records are presented below.

PERONOSPORA **cakiles** Savile sp. nov.

Sporangiophori amphigeni, 1–3 in stomate, 230–275 μ alt., 10–23 μ lat., hyalini, supra tertias vel medias quinquies ad septies ramosi; ramis valde divaricatis quorum majores alternantes, minores $\pm$ dichotomi, apicibus $\pm$ sigmoideis et 4–22 (–25) μ long. Sporangia 23–34 $\times$ 16–22 (–23.5) μ, hyalina, interdum ad basas apiculata. Oogonia et oospora mihi ignota sunt.

On leaves of *Cakile edentula* (Bigel.) Hook. var. *edentula*, salt marsh near Tracadie, Antigonish Co., N.S. (Savile 3262, 14 Aug. 1956, TYPE); North Sydney, Cape Breton Co., N.S. (Savile 3292); Notre Dame du Portage, Riviere du Loup Co., Que. (A. Payette 890); L'Islet Co., Que. (H. A. C. Jackson). All specimens in DAOM. Isotype distribution: BPI, FH, IMI, TRT, UPS.

Mr. A. Payette first collected this mildew in eastern Quebec in 1949 and a search of the phanerogamic herbarium yielded a second fragment from the same area. The type collection was fortunately made soon after sunrise, in heavy dew, and is in excellent fruit. All four specimens agree closely in morphology. It is with some trepidation that I add to the substantial

[1]Manuscript received November 9, 1956.
Contribution No. 1574 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

number of species of *Peronospora* described on members of the Cruciferae; but I am unable to match the present fungus from any published description or available specimens. *Cakile* is a rather unusual genus with no very close relatives. The closest genus known to bear a downy mildew is *Raphanus*, but the fungus on this plant, which is usually ascribed to *P. brassicae* Gäum., has substantially smaller sporangia in the specimens examined, although they tend to be somewhat larger than those on *Brassica* spp. The infected leaf tissues may become blackened by the accumulation of tannins or other materials, but no oospores have been found.

TAPHRINA TOSQUINETII (West.) Magn. Hedw. 29 : 23–25. 1890.

This species is recorded by Mix (8) from New Hampshire on *Alnus crispa* (Ait.) Pursh var. *mollis* (Fern.) Fern. I found it on the same host near St. Anthony, Nfld., in 1951 (det. A. J. Mix), and accordingly looked for it in Nova Scotia. It was found, again on *A. crispa* var. *mollis*, at Spry Bay, Halifax Co., and Corney Brook, 8 mi. northeast of Cheticamp, Inverness Co. These mid-August collections are in meager fruit and the fungus should be sought in early or mid-July. On this host the fungus is systemic and perennial in the specimens seen. There is no true witches' broom, but the leaves are small and often develop a marginal scorch, the appearance being that of a physiological disorder rather than a *Taphrina*. It is easily overlooked until once recognized. For discussion of the authority for this fungus see Bond (2).

STIGMATEA ROBERTIANI Fr., Summa Veg. Scand. p. 421. 1849.

Typical material was seen on *Geranium robertianum* L. at Corney Brook, Inverness Co. Lack of necrotic lesions and the small size of the fruit body make this fungus inconspicuous, but I have seldom seen an extensive colony of the host from which it was absent.

ERYSIPHE CICHORACEARUM DC., Fl. Fr. 2 : 274. 1805.

Infection was heavy on *Plantago oliganthos* R. & S. var. *fallax* Fern., in a salt marsh at Mahoney Beach, near Antigonish, Antigonish Co. (Savile 3295). It is reported by Wehmeyer on the ubiquitous *P. major* L. and on the sea-cliff species *P. juncoides* Lam. var. *decipiens* (Barn.) Fern. The latter was also found infected at Corney Brook and Cheticamp I., Inverness Co.

PHYLLACHORA GRAMINIS (Pers. ex Fr.) Fckl., Symb. Myc. 216. 1869.

A moderately heavy infection was found on *Agropyron trachycaulum* (Link) Malte at Corney Brook, Inverness Co. The organism is recorded by Wehmeyer on *A. cristatum* and *A. repens*.

COLEOSPORIUM CAMPANULAE (Pers.) Lév., Ann. Sci. Nat. Bot., III, 8 : 375. 1847.

Wehmeyer reports this rust on *Campanula rapunculoides* from Hants Co. and Annapolis Co. It was heavy on *C. rotundifolia* L. along the sea cliffs on Cheticamp I. and at Corney Brook Inverness Co. We have specimens on this host from Wis., Ont., Que., and Nfld., but not from western Canada.

The rust is also apparently unreported from the southern part of the range of the host. The race attacking *C. rotundifolia* may have reached North America in postglacial times and not yet had time to spread through the full range of the host.

COLEOSPORIUM SOLIDAGINIS (Schw.) Thüm., Bull. Torrey Bot. Club, 6 : 216. 1878.

Heavy on *Solidago sempervirens* L. in the salt marsh at Caribou I., Pictou Co., N.S. The host is not listed by Wehmeyer but it may be noted that we have a recent collection on it from Brackley Beach, P.E.I.

PUCCINIASTRUM POTENTILLAE Kom., in Jacz.-Kom.-Tranz. Fungi Rossiae 327. 1900.

Light infections were found on *Potentilla tridentata* Ait. at Presqu'ile, Inverness Co., and at Ecum Secum, Guysborough Co. This rust is reported only from New Brunswick by Wehmeyer, but it is much commoner than the dearth of published records suggests. The sori are hypophyllous and inconspicuous. We have specimens from various localities in Ont. Que., P.E.I., and Nfld., and the species seems to occupy at least all the eastern part of the range of its host.

PUCCINIA CALCITRAPAE DC., Fl. Fr. 2 : 221. 1805.

Puccinia cirsii Lasch, Rab. Fungi Eur. 89. 1859. Not *P. cirsii* Kirchner, Lotos 6 : 181. 1856. (= *P. punctiformis* (Str.) Röhl.).

P. centaureae DC., Fl. Fr. 5 : 59. 1815.

P. bardanae Cda. Icon. Fung. 4 : 17. 1840.

The above is the name selected by Hylander, Jørstad, and Nannfeldt (5) for the complex on Cynareae that has commonly been referred to the names listed here in synonymy. There is a little morphological variation from host to host, as much perhaps within *Centaurea* as between genera, and it should eventually be possible to break the species down into several varieties.

This rust was found on *Cirsium muticum* Michx. at Corney Brook, Inverness Co. Our only eastern record of a member of this complex on *Cirsium* is from Ste. Anne de la Pocatière, Que., also on *C. muticum*. The rust on *Arctium* (*P. bardanae*) is recorded from Nova Scotia.

PUCCINIA PRAEGRACILIS Arth., Bull. Torr. Bot. Club, 34 : 585. 1907, var. **cabotiana** var. nov.

Uredinia amphigena, minuta. Urediniosporae 16.5–22 × 14.5–19 μ; episporio 0.8–1.2 μ crass., subhyalino vel pallide flavo, leniter echinulato, poris indetectis. Telia amphigena, minuta, compacta, primo epidermide tecta. Teliosporae (33–)37–56(–61) μ (appendiculis inclusis) × 11.5–18 μ (appendiculis exclusis), appendiculis apicalibus 2.0–8.0 μ long., plerumque 3–5 in teliospora; episorio baso 0.6–1.0 μ crass., ochraceo, sumino 3.0–5.0 μ, appendiculis exclusis, castaneo; pedicello breve, ochraceo, firmo.

On leaves of *Hierochloë odorata* (L.) Beauv. at Sugar Loaf, 46° 57′ N.: 60° 28′ W., Victoria Co., N.S. (Savile 3291, 17 Aug. 1956, TYPE). Spent aecia, indistinguishable from those of the other varieties, occurred on contiguous *Habenaria psycodes* (L.) Spreng. (Savile 3290). Sugar Loaf, famous as the landfall of John Cabot on 24 June 1497, is on Aspy Bay just north of the northernmost point on the Cabot Trail. The site of the rusted plants was a fresh to slightly brackish marsh behind a barrier beach and close to a tidal pond. Isotype distribution: BPI, FH, IMI, MICH, O, PUR, TRT, UPS.

The existence of one or more varieties of *Puccinia praegracilis* in eastern Quebec and Cape Breton I. has been realized for several years, since the aecial stage was found on *Habenaria psycodes* near Riviere Ouelle, Kamouraska Co., Que., and on *Habenaria* sp. in southwest Cape Breton I. (9, 10). At that time I suspected that the latter host was *H. lacera*, but I now realize that *H. psycodes* is by far the commonest species in eastern Nova Scotia and I believe it to be the host of the earlier specimen. Finding the rust at Sugar Loaf climaxed a considerable search, conducted more in bog than in marsh habitats because the former was recorded as the habitat of the earlier collection. The grass was completely vegetative, consisting of rosettes of long slender leaves arising from extensive rhizomes; the flowering stems of this vernal species had disintegrated beyond recognition. Fortunately Dr. W. G. Dore was able to identify it with certainty, both from its morphology and from the scent, which was still detectable in the dried specimens. Intact plants from the rusted collection have been preserved in DAO.

A week later, under the guidance of Mr. J. G. A. Hamel, who was familiar with it, I visited the site of Mr. A. Payette's collection near Riviere Ouelle. Unfortunately all the *Habenaria* plants had vanished, in part at least through visitors to the beach digging them up for garden plants, and no rust could be found. Ecologically, however, the site proved to be almost identical with that at Sugar Loaf, and I do not doubt that *Hierochloë odorata*, which is common along the shore above and below Riviere Ouelle, is present.

It may be noted that the teliospores in this variety are longer than in either var. *praegracilis* or var. *connersii*, approaching those of *P. coronata*, but it shares with them the thin and pale urediniospore walls with invisible pores. It is of some interest to note that the several varieties seem to be as narrowly restricted in their aecial as in their telial hosts.

PUCCINIA SEYMOURIANA Arth., Bot. Gaz., 34 : 11. 1902.

Infected plants of *Spartina patens* (L.) Muhl. were found at Mahoney Beach, near Antigonish, Antigonish Co. (Savile 3293). The species is reported by Wehmeyer from Middleton, Annapolis Co., on *S. pectinata* Link and *Apocynum androsaemifolium* L. *S. patens* does not seem to have been previously recorded as a host.

UROMYCES ERVI West., Bull. Acad. Roy. Belg., 21, 2 : 234. 1854.

A heavy but very localized infection was found on a beach near Tracadie, Antigonish Co., 14 Aug. 1956 (Savile 3258), on *Vicia hirsuta* (L.) S. F. Gray (*Ervum hirsutum* L.), a delicate vetch that is scatteringly introduced on the Atlantic coast. The rust is chiefly distinguished from *U. fabae* in having repeating aecia. The secondary aecia, unaccompanied by pycnia, may be evenly scattered over all but the uppermost leaves, suggesting a systemic infection; in fact, I thought at first that the Tracadie specimen might be *Uromyces coloradensis*. The uredinia are very scarce in this specimen and in the few others examined, and they are apparently being superseded by the repeating aecia in the evolution of the species. The urediniospores and teliospores are not appreciably different from those of *U. fabae*. The urediniospore pores are frequently described as 2 or 2–3; but they are (2–)3–4 in Sydow Myc. Germ. 1312 and 3–4 in the present collection, and the character thus seems to be somewhat variable. This seems to be the first report of this species in North America.

UROMYCES JUNCI-EFFUSI Syd., Monog. Ured. 2 : 290. 1910.

Arthur (1) and Wehmeyer quote Fraser as reporting this rust on *Juncus filiformis* L. from Pictou, N.S., but it may be noted that the Fraser collections also include three from Nova Scotia on *J. effusus* L., one from Pictou, and two with no locality given. A collection was made near Tracadie, Antigonish Co., on *J. balticus* Willd. (Savile 3261). *U. junci-effusi* seems to be rare on this host. Neither Arthur nor Weiss and O'Brien (13) record it. One specimen in DAOM from California (Eureka, J. P. Tracy 8375) so labelled, proved to be *U. junci*, the common rust on *J. balticus*.

CERCOSPORA PONTEDERIAE Ell. & Dearn., Can. Rec. Sci. 5 : 270. 1893.

This parasite of *Pontederia cordata* L. is one of those inconspicuous fungi that prove to be much commoner than they were thought to be once one has seen them and knows what to look for. Until recently *Cercospora pontederiae* was known only from Texas, Wisconsin, and the type station at Niagara-on-the-Lake, Ont. In 1955 Mr. J. A. Parmelee and I found it in Bruce Co., Ont., and later, with little difficulty, found light infections in Algonquin Park and east of Ottawa. Three stands of *Pontederia cordata* in Nova Scotia were examined and trace infections were found in two: near Rhodes Corners, Lunenburg Co. (Savile 3243), and 4 mi. north of Bedford, Halifax Co. (Savile 3245). As Chupp (3) and others have remarked, the conidiophores are generally so pale that the species might equally well be placed in *Cercosporella*.

Isariopsis bulbigera (Fckl.) comb. nov.

Scolecotrichum bulbigerum Fckl., Symb. Myc. 106. 1870.

Ovularia bulbigera (Fckl.) Sacc., Syll. Fung. 4 : 140. 1886.

This fungus, which causes a leaf blotch of *Sanguisorba*, has been reported in North America only from Alaska. It was found in 1954 near Barkerville, B.C., on *Sanguisorba sitchensis* C. E. Mey. (Calder, Savile, and Ferguson

14318). Both it and *Marssonina sennenis* (see below) fruit very inconspicuously and the lesions appear sterile under the hand lens. Forewarned by previous experience, I took two collections of necrotic leaves of *S. canadensis* L. in Inverness Co., N.S., and one, from a bog near French Mtn. at 1400 ft. (Savile 3285), proved to bear this fungus. A composite description from the two Canadian specimens and Petrak Myc. Univ. 279 follows. Conidiophores hypophyllous, firmly united into synnemata of very variable size, *ca.* 60–360 μ high and 14–30 μ wide, septate, *ca.* 1.6–2.2 μ wide in the column but somewhat wider in the apical free portions, with 3–5 denticulate spore scars in the Nova Scotia collection, but usually 8–12 in that from British Columbia; the larger synnemata such as predominate in the Nova Scotia specimen are often distinctly fuscous, but in the other two collections they are generally hyaline except at the base. Mature, detached conidia *ca.* 11–20.5 × 4.5–10 (–12.5) μ, hyaline, continuous, subcylindric to ovoid, solitary (or rarely catenulate in Nova Scotia specimen), closely and finely echinulate with spines usually 0.5–1.0 μ high.

The generic disposition of this fungus presents some difficulty. It is not a typical *Ovularia*, apart from the fact that the genus *Ovularia* cannot be maintained (see below under *Ramularia pusilla*). The genus *Ramulaspera* Lindr. (Acta Soc. Faun. Flor. Fenn. 22, 3 : 5 1902) was set up to accommodate *Ovularia*-type fungi with echinulate spores. Examination of a specimen (Petrak Myc. Gen. 1256) of *R. salicina* (Vest.) Lindr., the type species, shows it to have fascicled, hyaline, denticulate conidiophores of the *Ramularia* type, and spores that are elongate-ovoid or occasionally fusiform, hyaline, borne singly or occasionally sparingly catenulate, continuous or rarely 1-septate, minutely echinulate. From it the present fungus is chiefly distinguished by its firm synnemata and frequent development of considerable pigment in the conidiophores. The type species of *Isariopsis* Fres. (Beitr. 3 : 87. 1863) is *I. alborosella* (Desm.) Sacc. (Fungi Ital. Tab. 838. 1881) (*Stysanus alborosellus* Desm., Ann. Sci. Nat., Ser. 3, 20 : 217. 1853. *Isariopsis pusilla* Fres., Beitr. 3 : 87. 1863). In this species the conidiophores are hyaline and are compacted into firm synnemata of slender hyphae *ca.* 2.5 μ wide that swell in the free apical portion to *ca.* 3.5 μ and bear several denticulate scars. The spores are more or less cylindric, hyaline, mostly 1-septate, smooth, and borne singly. The present organism is very close to *I. alborosella* in structure of the synnemata and conidiophores and in the generally solitary spores. It differs in the lack of a septum (a highly variable character in many species of the related genus *Ramularia*), in the echinulate spore walls, and in the frequent production of pigment in the conidiophores. The pigmentation seems to be at least in part a reflection of the amount of degradation of the host tissues. Thus it is much more pronounced in the Nova Scotia specimen, in which there are large, diffuse, and highly necrotic lesions, than in that from British Columbia, in which the spots are small and less necrotic. It will be remembered that many of these fungi with hyaline conidial states produce, under purely saprophytic growth, heavily pigmented perithecia. Echinulation

is so variable a character in many seemingly natural genera of fungi that I hesitate to put much emphasis upon it above the specific level. It has accordingly seemed best to place the *Sanguisorba* parasite in *Isariopsis*, the only satisfactory alternative being to erect a new genus for it.

RAMULARIA OXALIDIS Farl., Appalach., 3 : 251. 1884.

This species was heavy on *Oxalis montana* Raf. on French Mtn., Inverness Co., at 1400 ft. (Savile 3282) and at Musquodoboit Harbour, Halifax Co. (Savile 3241). It seems to be unrecorded from the Maritime Provinces. We have specimens in DAOM from three stations in Quebec, two from the lower St. Lawrence and one from near the summit of Mt. Orford, on the same host. It is apparently favored by high humidity and should be sought along the south shore of Nova Scotia where fog is frequent.

RAMULARIA PUSILLA Unger, Exanthema, 169. 1833.

Ovularia pusilla (Unger) Sacc., Fungi ital. t. 970. 1881.

Ramularia pulchella Ces. apud Rab., Herb. Viv. Myc. 1781. 1853.

A trace infection was found at Ecum Secum, Guysborough Co., on *Anthoxanthum odoratum* L., mixed with *Puccinia poae-nemoralis* Otth. I find no record of this fungus on *Anthoxanthum* and it may only attack tissues of this host that are senescent or have been weakened by rust attack. The fungus has not been previously reported from the Maritime Provinces.

The above name is clearly obligatory, as has been shown by Hughes (4), who gives the full synonymy. Briefly, the situation is that *R. pusilla* is the type species of *Ramularia*. As time went by *Ramularia* became the repository not only for species with solitary, ovoid spores, but also for those with catenulate, more or less cylindric, and often septate spores. Saccardo deemed it advisable to divide the genus, but unhappily, when he set up the segregate genus *Ovularia* (Mich. 2 : 17. 1880), it was so defined that it included the type of *Ramularia*. All typical *Ovularia* species must accordingly be put in *Ramularia*, and, if a separation is desired, it is the catenulate-spored species that must be removed. Saccardo further confused the issue when he emended *Ovularia* (Syll. Fung. 4 : 139. 1886) to include continuous-spored species with the spores in chains. Actually, in view of the increasing number of species in the *Ovularia–Ramularia–Cercosporella* complex that are proving to be polymorphic, there is some doubt as to whether such a division is warranted.

RAMULARIA RUBELLA (Bon.) Nannf., Lundell & Nannf. Fungi Exsic. Suec. 1992. 1950.

Crocysporium rubellum Bon., Bot. Zeit. 19 : 201. 1861.

Ovularia rubella (Bon.) Sacc., Syll. Fung. 4 : 145. 1886.

Ovularia obliqua (Cke.) Oudem., Hedwigia, 22 : 85. 1883.

Peronospora obliqua Cke., Microscop. Fungi, 160. 1865.

Ramularia obliqua (Cke.) Oud., Ned. Kruidk. Arch. 2, Ser. 1 : 262. 1872.

Ovularia monosporia (West.) Pound & Clem., Minn. Bot. Stud. 8 : 653. 1897.

Ovularia monosporia (West.) Sacc., Syll. Fung. 22 : 1296. 1913.

Ovularia monosporia (West.) Keissl. in Zahlbr., Ann. k.k. Naturhist. Hofmus. Wien, 29 : 462. 1914.

Oidium monosporium West., Bull. Soc. Roy. Bot. Belg. 2 : 252. 1863.

Ramularia obovata Fckl., Symb. 103. 1869.

Ovularia obovata (Fckl.) Sacc., Fungi Ital. t. 972. 1881.

Ovularia canaegricola (P. Henn.) P. Henn. in Sacc., Syll. Fung. 14 : 1055. 1899.

Ovularia obliqua (Cke.) Oud. var. *canaegricola* P. Henn., Notizbl. Königl. Bot. Gart. u. Mus. Berlin, 7 : 238. 1897.

Ramularia rumicis Kalchb. & Cke., Grev. 9 : 23. 1880.

Ramularia decipiens Ell. & Ev., J. Mycol. 1 : 70. 1885.

Ramularia circumfusa Ell. & Ev., Proc. Acad. Phil. 437. 1895.

Ramularia rumicis-scutati Allesch., Allesch. & Schnabl F. Bav. 695. 1897.

Any mycologist who studies the leaf-spotting fungi that attack *Rumex* subgen. *Lapathum* must be disturbed by the way in which the fungus in a given colony seems to change from season to season, by the multiplicity of *Ovularia* and *Ramularia* species described from these plants, and by the fact that few specimens fit very clearly into any one of these species. Weiss and O'Brien (14) suggest a considerable amount of lumping in this complex, and recent experience suggests that the whole group is actually a single pleomorphic species.

No hyphomycetes were recorded on *Rumex* by Wehmeyer, but a specimen on *R.* ? *crispus* L. was collected in Kings Co. by Mr. D. W. Creelman in 1951 and assigned at that time to *Ovularia obliqua*. On 16 Aug. 1956, a heavily spotted colony of *R. obtusifolius* L. was found near the Cabot Trail at the crossing of the North Aspy R., Victoria Co. (Savile 3289). Although the catenulate phase was predominant, many lesions bore both slender, catenulate, and occasionally 1-septate spores 8–28 × 1.8–2.8 μ, and broad, ovoid, solitary, and continuous spores (17–) 19–25 (–32) × (6.5–) 7.5–9.5 (–11) μ. Both spore types were on indistinguishable conidiophores and there can be little doubt as to their genetic connection. It thus seems that the most slender-spored form may transform under appropriate conditions to the broadest-spored form of the recognized series. This transformation is partly explained by a phenomenon seen in various species of *Ramularia*, wherein long, slender conidiophores, narrower than most of the conidia, will belatedly fragment into spores.

No authentic material of *Ramularia rubella* on the type host (*Rumex aquaticus* L.) has been available for study, and it was somewhat meagerly described, but the only real point of distinction between it and what has commonly been called *Ovularia obliqua* seems to be the reddish coloration of the conidia and conidiophores. Taking up of anthocyanins from the host cells occurs erratically in several species of *Ramularia*, e. g. *R. rosea* (Fckl.) Sacc. and *R. veronicae* Fckl. In the latter species it has been used as a taxonomic character; but on a single *Veronica* leaf I have seen, not only hyaline and red-tinged clusters of conidiophores, but enough variation in morphology to cover all the "species" of *Ramularia* and *Ovularia* described from this host genus.

Various authors have believed *Mycosphaerella rumicis* (Desm.) Lindau to be the perfect state of what has been called *Ovularia obliqua*; but Laibach (7) declares it to be a distinct fungus which be named *Ovosphaerella lapathi*. *Ovosphaerella* was set up to segregate those species of *Mycosphaerella* with conidial states in *Ovularia*, to correspond with *Ramularisphaerella* Kleb., whose conidial states are in *Ramularia*, and which Laibach (6) wantonly changed to *Ramosphaerella*. In view of the mutability of some of the conidial states such splitting of the perithecial states seems to have little value. Because of the difference of opinion as to the ascomycetous state it seems advisable to continue to use the appropriate name for the conidial state until the matter is settled.

Ramularia subsanguinea (Ell. & Ev.) comb. nov.

Cercospora subsanguinea Ell. & Ev., J. Mycol. 4 : 4. 1888.
Ramularia rubicunda Bres., Hedw. 35 : 200. 1896.

Several authors have noted that *Ramularia subsanguinea* is the correct name for this leaf-spotting parasite of *Maianthemum*, but none seems to have made the combination. This seems to be an appropriate opportunity to validate the name. A small collection was made on *M. canadense* Desf. on French Mtn., Inverness Co. (Savile 3283). It agrees well with specimens in DAOM on the same host from Manitoba and Quebec, and with two on *M. dilatatum* (Wood) Howell, including isotype material, from British Columbia.

MARSSONINA SENNENIS Gonz. Frag., Trab. Mus. Nac. Cienc. Nat. Madrid, Ser. Bot. 9 : 90. 1916.

Shaw and Sprague (11) recently reported this foliage parasite of *Sanguisorba* from Alaska, and we took it in British Columbia in 1954 (Calder, Savile, and Ferguson 15009B). A collection of a seemingly sterile leaf spot on *S. canadensis* L. taken at Corney Brook, Inverness Co., N.S. (Savile 3274) bore this fungus in sparse fruit. The epiphyllous acervuli are almost concolorous with the necrotic leaf tissue and are difficult to see even under the dissecting microscope. The spores were 15.5–25 × 4.7–6.5 μ, compared with 20–28 × 3–7 μ in the original description, 17.5–28 × 3.5–7 μ given by Shaw and Sprague, and 22–31 (–35) × 4.5–7.2 μ in the British Columbia collection. As Shaw and Sprague have noted, the conidiophore tip may remain attached to the spore.

SEPTOGLOEUM POTENTILLAE Allesch., Ber. Bayer. Bot. Gesellsch., 4 : 38. 1896.

What is believed to be this fungus was taken on *Potentilla tridentata* Ait. on Cheticamp I., Inverness Co. (Savile 3270). Spores were 24–34 (–37) × 4.8–6.0 μ, hyaline, 3-septate, rounded at apex, and subtruncate below. The acervuli were 150–200 μ diam., usually more or less ostiolate at first but becoming open later.

SEPTORIA LEVISTICI Westd., Herb. Crypt. Belg. 935. 1854 (fide N.A. Fl. 7 : 1096); Bull. Acad. Roy. Belg., ser. 2, t. 2, no. 7: p. 31 (in reprint). 1857. *Septoria ligustici* Guba, Rhodora, 41 : 519. 1939.

A leaf spot on *Ligusticum scothicum* L. at Merigomish I., Pictou Co., yielded pycnidia 100 μ diam. or less, containing acicular spores 20–33 × 1.0–1.4 μ, hyaline, apparently continuous. *Septoria levistici* on *Levisticum officinale* W. D. J. Koch (*Ligusticum levisticum* L.) was described as having spores 30–40 × 1.1 μ and minute pycnidia. D. Sacc. Myc. It. 557, on the type host, yields spores 15–35 × 1.0–1.5 μ, continuous and acicular, and the pycnidia are as above. *S. ligustici* was described as having spores 12–22 (–28) × 1.5 μ and pycnidia 60–100 μ. It does not seem to be morphologically distinct from D. Sacc. Myc. It. 557, and, in view of the close relationship of the host genera, probably should not be maintained as a separate species. No *Septoria* on *Ligusticum* has been reported previously from Nova Scotia. Only a few infected leaves were found, but the region was in the periphery of a moderating hurricane at the time, and collecting conditions in the unsheltered salt marsh were difficult. The fungus may have been relatively abundant.

Acknowledgments

I am indebted to Dr. B. Boivin for assistance in the Latin diagnosis of *Peronospora cakiles*; to Dr. S. J. Hughes for advice on the nomenclature and disposition of some of the Hyphomycetes; and to Dr. R. W. G. Dennis and Dr. R. L. Steyaert for bibliographic assistance.

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THE EFFECT OF FUSARIUM TOXINS ON THE ENZYMIC ACTIVITY OF TOMATO HYPOCOTYL MITOCHONDRIA[1]

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Abstract

The presence of an active cyclophorase system capable of oxidizing the principal acids of the Krebs acid cycle was demonstrated in mitochondria from hypocotyls and cotyledons of tomato seedlings. The optimum conditions for active mitochondrial preparations and maximum enzyme activity, especially for the succinoxidase system, were determined and are reported.

Lycomarasmin and fusaric acid, two toxins produced by *Fusarium oxysporum* f. *lycopersici*, inhibit the succinoxidase and cytochrome oxidase activity of the mitochondria at a concentration of 10^{-3} *M*. The inhibition was overcome by the addition of catalytic amounts of cytochrome *c*. No effect was observed on succinic dehydrogenase. It was concluded that the toxins inhibit enzyme activity by affecting the integrity of the mitochondria allowing diffusion of cytochrome *c* from active sites. The role of the toxins in *Fusarium* wilt of tomatoes is discussed.

Introduction

Among theories advanced to explain the mechanism of wilting of tomato plants invaded by *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder & Hansen (*F. lycopersici*), the "vessel-plugging" theory and the "toxin" theory seemed to the present investigators to be the most plausible. The former theory, which involves the plugging of vessels by mycelium and its metabolic products within the host tissue, appeared to be more acceptable (4, 14, 22). On the other hand, most investigators agree that toxins secreted by the pathogen may be important as secondary causal factors in initiating symptoms of the disease (3, 5, 24).

Lycomarasmin was the first toxic substance isolated in crystalline form from cultures of *F. lycopersici* and is specifically produced by this organism (19). It has an empirical formula of $C_9H_{18}O_7N_3$ and is a tripeptide yielding on hydrolysis one molecule each of ammonia, glycine, aspartic, and pyruvic acids (20, 21). When absorbed by tomato cuttings, the toxin has been claimed to induce some of the symptoms of the disease, such as progressive yellowing, browning, and drying of leaves; dark patches on foliage; flaccid state and wilting of stems, petioles, and leaves. Gaümann and Jaag (6) explain the toxic action of lycomarasmin by a destruction of the semipermeability of the plasma membranes of host cells and consequent excessive transpiration. However, it has been demonstrated that naturally infected plants show a continuous decline in transpiration immediately preceding or during wilting (22).

[1]Manuscript received September 19, 1956.

Joint contribution from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario (Contribution No. 1565), and the Department of Botany, McGill University, Montreal, Quebec. This paper constitutes part of a thesis submitted by the senior author to the Faculty of Graduate Studies and Research, McGill University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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The second more recently isolated toxin in crystalline form is fusaric acid, not specifically produced by *F. lycopersici.* It has an empirical formula of $C_{10}H_{13}O_2N$ and has been identified as 5-*n*-butyl-2-pyridin carboxylic acid or 5-*n*-butyl picolic acid, which has been synthesized (8, 25). Fusaric acid produces necrotic areas between the veins of leaf blades and streaks on stems of the tomato plant. Respiration of tomato tissue is inhibited 50% by this acid at a concentration of 5×10^{-3} *M* (17).

The present authors have investigated the mechanism of action of these two particular toxins to determine whether they may be specific inhibitors of certain enzymes controlling the respiration of plant tissues. The recent discoveries that plant mitochondria have the ability to catalyze the oxidation of pyruvic acid through the Krebs acid cycle provide a multienzyme system upon which the effect of toxins could be judged as to their action on the oxidative centers of respiration.

Accordingly, this paper reports on the isolation and some of the enzymatic properties of mitochondria from tomato hypocotyls as well as the effects of toxins thereon.

Methods and Materials

Seeds of tomato, *Lycopersicon esculentum* Mill. var. John Baer, were sterilized for 5 minutes with mercuric chloride (1 : 10,000) and after several washings were soaked in water for 1 hour. The seeds were sown in wet sterile vermiculite in a flat and placed in a dark room at 26° to 27° C. After 7 days, the etiolated seedlings attained a height of 6 to 8 cm.

Extraction of Mitochondria

Mitochondria were isolated by differential centrifugation following the procedure of Millerd *et al.* (15). All operations were conducted in the cold. Etiolated seedlings were cut at the base of the hypocotyls and the cotyledons removed. Generally 25 to 35 g. of hypocotyl tissue was washed with cold water and ground with sand and a buffer consisting of 0.4 *M* sucrose and 0.1 *M* phosphate and having a pH of 6.9 to 7.1, equal in volume to the weight of the tissue. The slurry was strained through muslin and the filtrate centrifuged twice for 5 minutes at 500 × *g*, the residue being discarded each time. The clear supernatant liquid was centrifuged at 10,000 to 12,000 × *g* for 15 minutes and after the supernatant fluid was drawn off, the sedimented pellet was resuspended in half the original volume of buffer and centrifuged once more for 15 minutes at 10,000 to 12,000 × *g*. Finally, the resulting pellet was resuspended in 1/10th the original volume of buffer. This fraction is designated as mitochondria. The particles were spherical in shape and varied in size from 0.5 to 1.0 μ, and stained vividly with Janus green B (1 : 10,000).

Enzyme Activity

The activity of mitochondria was measured by determining the oxygen uptake in the presence of the substrate and appropriate cofactors in Warburg respirometers shaken in a bath held at 30° C. The usual precautions and techniques described by Umbreit *et al.* (23) were followed. Except where

otherwise stated, the reaction system contained 300 μM sucrose, 50 μM phosphate (pH 7.0 to 7.1), 2 μM magnesium sulphate, 30 μM sodium succinate, 0.5 ml. of mitochondrial preparation in a final volume of 2.2 ml. and with 0.2 ml. of 2 N NaOH and filter paper in the well.

Activities were expressed as Q_{O_2} (N) calculated from the linear part of the progress curve of oxygen uptake. Nitrogen was determined by the micro-Kjeldahl method of Clark (1) as modified by Hiller *et al.* (10).

Succinic dehydrogenase activity was measured by the "Thunberg technique" (23). The substrate was placed in the hollow stopper of the tube and mixed with the buffered mitochondrial preparation containing methylene blue at zero time. Tubes were held in a bath at 30° C. and the reduction of methylene blue was followed in a Beckman DU spectrophotometer at 660 mμ to 90% decolorization.

Adenosine triphosphoric acid and its sodium salt were obtained from General Biochemicals Inc. Cytochrome *c* was obtained from Nutritional Biochemicals Co.

Samples of pure lycomarasmin and fusaric acid were kindly supplied by Dr. E. Gaümann, Zürich, Switzerland, to whom we are greatly indebted. Extraction of lycomarasmin from cultures of *F. lycopersici* was also attempted according to the procedure of Plattner and Clauson-Kaas (19). However, the procedure of purification was stopped after the first ethanol precipitation (18). The substance thus obtained contained impurities as shown by the yellow-brown color of the material, its low nitrogen content, and chromatographic analysis (20). Nevertheless, when absorbed by tomato cuttings, this material produced on stems and leaves symptoms similar to those described for pure lycomarasmin (7). In the text, this material is referred to as "crude lycomarasmin".

Experimental Results

Intracellular Localization

Succinoxidase activity was used in all preparatory experiments as a criterion of the enzymic activity of the mitochondria since it is one of the most labile enzyme systems. Table I shows that activity is greatest in particulate fractions deposited at 17,000 × *g* in this experiment. The low activity found in the

TABLE I

SUCCINOXIDASE ACTIVITY OF THE CELL COMPONENTS

Cell fraction	μl. O_2/hr./5 g. fresh weight
Homogenate (after filtration through cheesecloth)	200
Xylem + cell wall debris + nuclei (centrifuged at 500 × g/5 min.)	
Not washed	13
Washed	6
Mitochondria (twice centrifuged at 17,000 × g/15 min.)	105
Mitochondria (twice centrifuged at 17,000 × g/15 min.) + cytochrome *c* at 10^{-5} M	138
Supernatant (microsomes + cytoplasmic fluid)	0

cell debris and nuclear fraction is probably due to adsorption by these cellular elements. Centrifugation at 10,000 × *g* for 15 or 20 minutes was sufficient to deposit the larger part of the active material and this was enhanced if two low speed centrifugations instead of one were made prior to the high speed centrifugation.

Effect of Composition of the Extraction and Reaction Media

The activity of mitochondria isolated from tomato hypocotyls showed the same dependency on the tonicity of the medium as had been found with mung bean (15) and cauliflower (13). Greatest activity was found in mitochondria extracted from a medium containing 0.4 *M* sucrose as compared with higher and lower concentrations. However, there was always some activity when sucrose was omitted.

The presence of cytochrome *c* had no effect on the rate of oxidation of succinate provided the mitochondria were prepared in the cold under the conditions described. Both manganese and magnesium ions accelerated the rate of oxidation, the latter being slightly more effective (Table II).

TABLE II

EFFECT OF SOME COFACTORS ON SUCCINATE OXIDATION

System	Q_{O_2} (N)	
	I	II
No additions	0	0
+ Succinate 30 μM	261	193
+ Succinate + cytochrome *c* 0.01 μM	269	—
+ Succinate + $MnSO_4$ 2 μM	—	243
+ Succinate + $MgSO_4$ 2 μM	—	269

Succinoxidase Activity of Hypocotyls and Cotyledons

Mitochondria prepared from hypocotyls alone invariably showed greater enzymic activity per mg. N than those from the whole aerial portion of the seedlings (Fig. 1). The nitrogen content of the hypocotyl preparations was also more uniform, varying between 0.6 to 0.8 mg. N per ml. in comparison to values ranging from 0.4 to 1.0 mg. per ml. in the latter preparations. There was little or no endogenous oxygen uptake in hypocotyl preparations as compared to values of Q_{O_2} (N) between 50 and 100 when cotyledons were included. The lower succinoxidase activity and the higher rate of endogenous oxygen uptake of preparations from the whole aerial tissue may be due to a lecithinase present in the cotyledons and not in the stem portion. Kates (11, 12) showed that lecithinase is associated with the plastid fraction in plant cells and therefore should be more abundant in cotyledons. Goodwin and Waygood (9) have shown that a lecithinase in mitochondrial preparations from barley seedlings destroys succinic dehydrogenase and provides substrate for lipoxidase resulting in a greater endogenous oxygen uptake than if the lecithinase was inhibited.

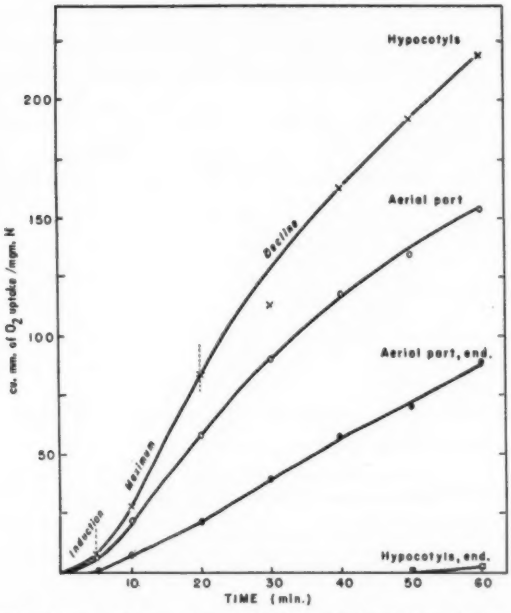

FIG. 1. Succinoxidase activity of mitochondria obtained from different portions of tomato seedlings. Aerial part includes hypocotyls and cotyledons. End. = endogenous oxygen uptake.

The progress curve of oxygen uptake by hypocotyl preparations showed three phases beginning with a lag which lasted approximately 5 minutes, followed by 15 to 20 minutes of maximum oxygen uptake which subsequently declined (Fig. 1). The cause of the lag period was not investigated, but all Q_{O_2} (N) values were calculated from the mean maximum slope of the curve.

Malonate competitively inhibited the oxidation, inhibition being complete at an equimolar concentration. Saturation of the oxidase was attained by succinate concentrations of 0.03 *M* or greater.

The cytochrome oxidase component of the system was demonstrated by using hydroquinone as substrate and its oxidation was augmented by the addition of cytochrome *c*. Succinic dehydrogenase activity was measured by the "Thunberg technique" and complete decolorization of methylene blue was attained in less than half an hour with substrate. No decolorization of methylene blue occurred in the absence of substrate or when the enzyme was boiled for 1 minute prior to use.

Oxidation of Other Krebs Cycle Acids by Tomato Mitochondria

Several acids known to be the intermediates in the Krebs cycle oxidations were oxidized at varying rates by the tomato mitochondria, and data assembled in Table III show that the rates were slightly greater than with mung bean mitochondria (15). The presence of adenosine triphosphate (ATP) accelerated

TABLE III

OXIDATION OF THE KREBS CYCLE ACIDS AND EFFECT OF ATP

Krebs cycle acid	Concentration, μM	Q_{O_2} (N)		
		Acid alone	Acid + ATP 0.5 μM	Acid + pyruvate 20 μM
None	—	0	0	0
Pyruvate	20	0	—	—
Citrate	20	106	111	—
	2	31	—	68
l-Malate	20	40	67	—
	2	13	—	76*
Fumarate	2	16	—	57
α-Ketoglutarate	20	87	117	—
	2	33	—	73*
Succinate	20	162	165	—
	2	56	—	132

*System also contained 0.5 μM ATP.

the oxidation of α-ketoglutarate and *l*-malate, but had little or no effect on the rate of oxidation of the other acids. Although pyruvate alone was not oxidized by the particles, the oxidation of the other acids used at a catalytic concentration was increased by the presence of pyruvate (Table III). This would be expected if oxidation occurred via a pathway involving the condensation of pyruvate or a derivative.

From these experiments it was concluded that particles which were isolated by differential centrifugation from tomato hypocotyl tissue, and which are referred to as mitochondria, are capable of carrying on most, if not all, of the reactions of the Krebs acid cycle and appeared to be no different from those isolated from other plant tissues (15). Accordingly, the preparations were used as a test system upon which the effect of lycomarasmin and fusaric acid could be determined.

Effect of Lycomarasmin on the Enzymic Activity of Tomato Mitochondria

Owing to a limited supply, pure lycomarasmin was used at concentrations of 10^{-2} *M* or less. At concentrations of 10^{-3} *M* and below, lycomarasmin increased the rate of succinate oxidation by tomato mitochondria, but above this level, oxidation was inhibited to the extent of 50% at 10^{-2} *M*. The data are graphically represented in Fig. 2, the horizontal line showing the control value for succinate oxidation in the absence of lycomarasmin. Extrapolation of the curve shows that complete inhibition would probably occur at about 4×10^{-2} *M* lycomarasmin.

"Crude lycomarasmin" showed a greater effect on succinoxidase activity than pure lycomarasmin and caused complete inhibition of the enzyme activity at 10^{-2} *M* concentration (Table IV). However, the effect of different concentrations of the "crude lycomarasmin" on succinoxidase activity exhibited trends almost similar to that obtained with pure lycomarasmin (Fig. 2).

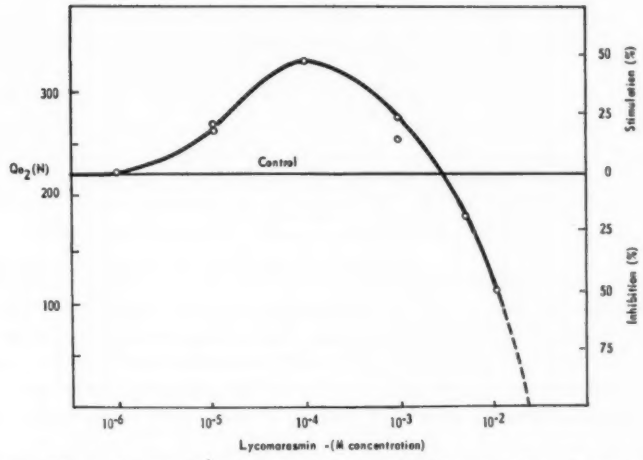

FIG. 2. Effect of lycomarasmin (Lyc.) on succinoxidase activity of tomato mitochondria. Maximum enzyme activity for the control is represented by the horizontal line.

Gaümann *et al.* (7) found that the complex iron–lycomarasmin was 10 times more toxic than lycomarasmin in initiating symptoms of its effects. However, the presence of ferric chloride ($FeCl_3$) at 10^{-3} *M* did not increase the inhibitory effect of "crude lycomarasmin" on succinoxidase activity at this concentration (Table IV).

TABLE IV

EFFECT OF "CRUDE LYCOMARASIN" AND FERRIC CHLORIDE ON SUCCINOXIDASE ACTIVITY OF TOMATO MITOCHONDRIA

System	Q_{O_2} (N)
No additions	0
+ Succinate 30 μM	219
+ Succinate + "crude lycomarasmin" 10^{-2} *M*	0
+ Succinate + "crude lycomarasmin" 10^{-3} *M*	142
+ Succinate + "crude lycomarasmin" 10^{-3} *M* + $FeCl_3$ 10^{-3} *M*	146

The inhibition caused by 10^{-2} *M* lycomarasmin was overcome completely by the addition of 2×10^{-5} *M* cytochrome *c* to the reaction system (Table V). Thus it is inferred that lycomarasmin interferes with the cytochrome *c* – cytochrome oxidase sequence of succinoxidase. The mechanism of its action may be nonspecific by affecting the integrity of the particulate material causing a "leaching out" of cytochrome *c* in the same way as do inadequate isolation procedures (15).

Effect of Fusaric Acid on Succinoxidase Activity

Succinoxidase activity was inhibited almost completely by 10^{-2} *M* fusaric acid. At concentrations of 5×10^{-3} *M* and 10^{-3} *M* the inhibition was 35% and 17% respectively after 1 hour. The addition of 10^{-5} *M* cytochrome *c*

TABLE V

EFFECT OF CYTOCHROME *c* ON LYCOMARASMIN INHIBITION OF SUCCINATE OXIDATION BY TOMATO MITOCHONDRIA

System	Q_{O_2} (N)
No additions	0
+ Succinate 30 μM + cytochrome *c* 2×10^{-5} *M*	228
+ Succinate + lycomarasmin 10^{-2} *M*	131
+ Succinate + cytochrome *c* + lycomarasmin 10^{-2} *M*	240

completely overcame the inhibition caused by 10^{-2} *M* fusaric acid (Fig. 3). The inhibition by fusaric acid resembles that brought about by lycomarasmin and it is concluded therefore that fusaric acid affects the succinoxidase system directly by interfering with the cytochrome oxidase system or indirectly by its action on the structural integrity of the particles. However, unlike lycomarasmin, fusaric acid did not stimulate enzyme activity at low concentrations.

Naef-Roth and Reusser (17) found that fusaric acid at concentrations of 2×10^{-2} *M*, 10^{-2} *M*, and 5×10^{-3} *M* inhibited the respiration of tomato tissue 100%, 80%, and 50% respectively. It is tempting to speculate that this effect may be due to its action on the cytochrome system.

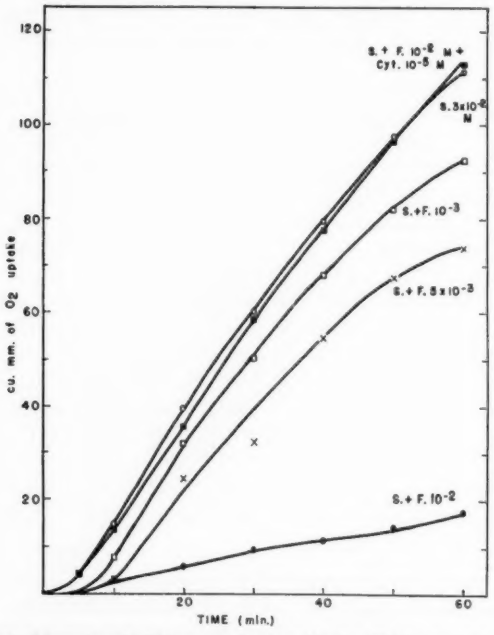

FIG. 3. Effect of fusaric acid (F.) on succinate oxidation (S.) by tomato mitochrondria. Cyt. = cytochrome *c*.

Effect of Lycomarasmin and Fusaric Acid on Cytochrome Oxidase

Using hydroquinone as substrate for demonstrating cytochrome oxidase activity it was shown that fusaric acid at a concentration of 10^{-2} *M* caused an 85% inhibition of oxygen uptake (Table VI). Lycomarasmin was less effective at this concentration, inhibiting only to the extent of 50%. However neither lycomarasmin nor fusaric acid caused any inhibition at this concentration in systems fortified with 2×10^{-5} *M* cytochrome *c* (Table VI). Thus, the addition of cytochrome *c* overcomes inhibition by either lycomarasmin or fusaric acid, indicating that they influence the reaction system at the cytochrome *c* stage in the sequence of reactions.

TABLE VI

EFFECT OF CYTOCHROME *c* ON LYCOMARASMIN AND FUSARIC INHIBITIONS OF CYTOCHROME OXIDASE ACTIVITY

System	Q_{O_2} (N)
Without substrate (H_2Q)	21
+ H_2Q (3 mg. of hydroquinone)	310
+ H_2Q + lycomarasmin 10^{-2} *M*	175
+ H_2Q + fusaric acid 10^{-2} *M*	45
+ H_2Q + cytochrome *c* 2×10^{-5} *M*	352
+ H_2Q + lycomarasmin + cytochrome *c*	352
+ H_2Q + fusaric acid + cytochrome *c*	384

Effect of Lycomarasmin and Fusaric Acid on Succinic Dehydrogenase

Since succinate dehydrogenation anaerobically in the presence of a hydrogen acceptor does not require the presence of the cytochrome – cytochrome oxidase system, no particular effect of lycomarasmin or fusaric acid must be expected, if these toxins affect only the cytochrome oxidase system. The reaction in this case was mediated by methylene blue and the toxins did not decrease the succinic dehydrogenase activity (Fig. 4), thus supporting the previous results that the toxins affect only the cytochrome *c* stage of the succinoxidase system.

The succinoxidase system of particulate material from plants is very labile and is one of the first to be inactivated during isolation by inadequate procedures and when the structural integrity of the particles is altered. It follows that the toxins do not poison the enzymes directly, but rather affect the integrity of the particles and cause the diffusion of the cytochrome *c* and/or a partial destruction of this cofactor.

Discussion

The presence of a cellular particulate system capable of oxidizing the principal substrates of the Krebs acid cycle was demonstrated in cotyledons and hypocotyl tissue from tomato seedlings. The particles stained vividly with Janus green B and appear to be identical with mitochondria (2).

The particulate material isolated from extracts of cotyledonous tissue was characterized by a higher endogenous rate of oxygen uptake than that from

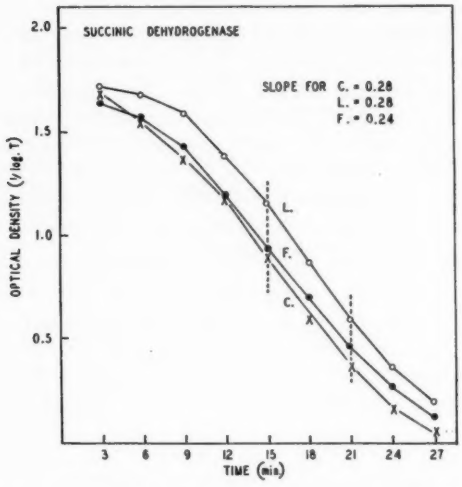

FIG. 4. Effect of lycomarasmin (L.) and fusaric acid (F.) at 2×10^{-2} *M* on succinic dehydrogenase activity. C. = control which contained in addition to the buffered mitochondrial preparation, 2 μM $MgSO_4$, 30 μM succinate, 1.0 ml. of methylene blue (1 : 10,000), and water to make up 5.0 ml.

hypocotyl tissue. It is suggested that the presence of more active lecithinase in the former tissue would initiate unsaturated fat oxidation by lipoxidase associated with the mitochondria.

The optimum conditions for the activity of the succinoxidase system have been determined and there appears to be little difference between the properties of mitochondria isolated from tomato hypocotyl tissue and those from mung bean (15, 16).

Lycomarasmin and fusaric acid produced by *F. lycopersici* in liquid cultures were found to inhibit succinoxidase and cytochrome oxidase activity of tomato hypocotyl mitochondria. Succinic dehydrogenase was not affected. Crude lycomarasmin was more inhibitory. Inhibition of succinoxidase and cytochrome oxidase was overcome by the addition of small amounts of cytochrome *c*. Since mitochondria when adequately prepared catalyze the oxidation of the Krebs cycle acids without requiring extraneous cytochrome *c*, it is concluded that these toxins inhibit enzyme activity by altering the structural integrity of the cytoplasmic particles, resulting in a diffusion of cytochrome *c* away from the active sites. These sites can be filled by the addition of more cytochrome *c*. A partial destruction of cytochrome *c* is also possible, thus requiring further supply of this cofactor for maximum enzyme activity.

Gaümann and Jaag (6) explain the toxic action of lycomarasmin on the cell by the destruction of the semipermeability of the plasma membrane, resulting in excessive transpiration and water absorption. However, Scheffer and Walker (22) demonstrated that the course of transpiration of naturally

infected plants and that of tomato cuttings dipped in solutions of lycomarasmin were different. Naturally-infected plants showed a decline rather than excessive transpiration in the period immediately preceding and during wilting.

Our results support the idea that lycomarasmin and fusaric acid could possibly affect host metabolism by removing cytochrome *c* from the active sites, thus causing disturbances in linked-enzyme systems rather than destroying the semipermeability of the plasma membranes. This theory provides a satisfactory explanation for the stimulating effects of low doses of lycomarasmin. The results would also explain the inhibition of tomato tissue respiration by fusaric acid, as found by Naef-Roth and Reusser (17).

However, in contrast to the preceding explanation, the following observations would show that lycomarasmin and fusaric acid are not involved in the wilting of tomatoes: (1) Concentrations of toxins required for the complete inhibition of the succinoxidase activity of tomato mitochondria appear to be too high for these toxins to be specific inhibitors. (2) The lycomarasmin inhibition of succinoxidase activity was not increased by the presence of free iron ($FeCl_3$), although it has been observed that the presence of free iron induces the formation of the complex iron–lycomarasmin, which is 10 times more toxic than pure lycomarasmin (7). Scheffer and Walker (22) showed that feeding increased iron solutions to infected plants did not change the intensity of wilt symptoms and the time of their manifestation, and they produced this as evidence against lycomarasmin participating in *Fusarium* wilt. Since lycomarasmin is an efficient chelating agent, there are many points at which it could affect host metabolism by chelating metal ions involved in redox couples. Among the metal ions, iron is most likely to be involved; it forms a ferric–lycomarasmin complex which could alter the normal equilibrium state of such couples.

Résumé

La présence d'un système enzymatique capable d'oxyder les principaux acides du cycle de Krebs a été nettement démontrée dans les mitochondries extraites des tissus (hypocotyles) des plantules de tomate. Les conditions optimum pour la préparation de mitochondries actives enzymatiquement furent étudiées et sont rapportées dans la présente communication.

La lycomarasmine et l'acide fusarique, deux toxines produites par *Fusarium lycopersici*, inhibent l'activité de la succinoxidase et de la cytochrome oxidase contenues dans les mitochondries de la tomate, mais sont sans effet sur la déhydrogénase succinique. Cependant, l'apport de cytochrome *c* annula l'action inhibitrice de ces toxines. Il est donc permis de conclure que ces toxines altèrent la structure des mitochondries, causent la destruction du cytochrome *c* lié à ces particules, et par voie de conséquence inhibent l'activité de la succinoxidase. Le rôle de ces toxines dans la maladie fusarienne de la tomate est aussi discuté à la lumière de ces résultats.

Acknowledgments

Revision and helpful criticism of the manuscript by Dr. C. Perrault, Officer-in-Charge, and by Dr. R. O. Lachance, and also material assistance by Mr. L. Dubé and other members of the staff, Science Service Laboratory, Ste. Anne de la Pocatière, Quebec, are gratefully acknowledged.

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STUDIES IN TREE PHYSIOLOGY

IV. FURTHER INVESTIGATIONS OF SEASONAL CHANGES IN MOISTURE CONTENT OF CERTAIN CANADIAN FOREST TREES[1]

J. CLARK[2] AND R. DARNLEY GIBBS[3]

Abstract

Complete seasonal patterns of moisture content of the wood and bark of *Betula lutea* (which resembles other species of birch) and of *Tsuga canadensis* are given, along with those for sapwood and heartwood of *Abies balsamea* and *Picea rubens*. Incomplete seasonal data are also presented on the moisture content of the wood of *Picea glauca* and *Thuja occidentalis*.

Minor variations in the water content pattern of yellow birch due to precipitation, evapotranspiration, and winter insolation are described. These variations, examined in the light of climatic data, lend little support to the view that birch dieback is due to an increase in temperature or to drought, except possibly through their effects on some unknown biotic factor.

The practical aspects of tree water content studies are discussed with particular reference to the questions of flotation and sinkage of logs.

Introduction

Some twenty-five years ago the late Prof. G. W. Scarth and one of us (R.D.G.) were asked to co-operate in work on forest trees designed to lessen the loss by sinkage of floated logs. This work involved a study of seasonal changes in the water content of untreated standing trees; studies of the effects of girdling upon the water content of standing trees; and measurements of changes in amount and distribution of water and gas in seasoning and/or floated logs, both untreated and treated in various ways. The results of this early work were published in a series of papers (16, 7, 29, 8, 9, 10, 11).

In the course of these studies it became clear that little was known of the seasonal changes in water content of standing trees in general and this so interested one of us (R.D.G.) that he continued to investigate trees and in particular to make regular measurements of seasonal changes in water content of as many species as possible, as well as to follow the changes in food reserves of one of them. Some of the results of this work were published in a further series of papers (12, 13, 14, 15).

From about 1932, it has been clear that in parts of Eastern Canada birch (2) and perhaps other trees (26) have been profoundly affected by some unknown agent or agents. In birch, the trouble—known as "birch dieback"—has resulted in the death of millions of individual trees. The malady appeared first in south central New Brunswick (2) and seemed to spread outwards until in 1945 it affected large areas of New Brunswick, Quebec, Prince Edward Island, Nova Scotia, and Maine (4).

[1]Manuscript received October 31, 1956.

Contribution No. 339, Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada, and McGill University, Montreal, Quebec. Based in part on a thesis by J. Clark presented in June, 1952, to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of Master of Science.

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The first studies of dieback were concerned mainly with the possibility of the cause being the bronze birch borer (*Agrilus anxius* Gory) or other insects. These studies resulted in the conclusion that insects were not the primary cause (2, 3). Similarly, studies of fungi and viruses failed to indicate any that were capable of initiating the condition (20, 19). Failure to demonstrate any clear biotic cause led to serious consideration of the hypothesis that dieback was a physiological disturbance resulting from climatic factors, particularly drought and possibly high temperatures. Several authors have presented evidence in support of this viewpoint (26, 21, 22) but it was not conclusive. This suggested to the present authors the need for a better understanding of the normal water economy of yellow birch and better methods of detecting the significance of precipitation deficiencies that would seriously affect the tree.

Since it has been shown that wire birch, *Betula populifolia* Marsh., and white (paper) birch, *B. papyrifera* Marsh., have low water content at the end of the winter and again at the end of the summer (7, 12), it was thought that an investigation of yellow birch, *B. lutea* Michx., would indicate similar "stress periods" which might be significant if the drought factor is indeed involved in dieback. The meteorological data were also studied using Thornthwaite's methods (32, 33), to see if these (by combining the effects of temperature and evapotranspiration on the water balance) would give a clearer indication of a period of unfavorable climate.

It has long been known that a form of dieback in fruit trees is due to a lack of certain microelements (zinc, copper, etc.) particularly on soils that are periodically very wet or very dry (30). This suggested to us the desirability also of investigating the microelement aspect of birch physiology, and in early 1950 a preliminary injection experiment involving 30 trees was carried out, with the kind help of Dr. D. A. Fraser, at the Petawawa Forest Experiment Station. At a later date, further work on this aspect of the problem was carried on as a co-operative research project at McGill University with financial assistance from the Canada Department of Agriculture. Unfortunately, this work has been suspended because of the lack of suitable assistants.

The early work on seasonal changes in moisture content of trees indicated very marked changes in hardwoods such as birch and poplar but small fluctuations in softwoods such as jack pine, *Pinus banksiana* Lamb.; white spruce, *Picea glauca* (Moench) Voss; and balsam fir, *Abies balsamea* (L.) Mill. (9). The analyses from the softwoods were so few, however, that it seemed desirable to extend the work. One of us (R.D.G.) was able, between 1936 and 1943 to cut and analyze 127 eastern hemlock, *Tsuga canadensis* (L.) Carr. The earlier part of this work has been reported elsewhere (9), and an outline of the total results has appeared (15), but it is here treated in detail as Section 3.2.

The Pulp and Paper Research Institute of Canada (P.P.R.I.C.) became interested and made available sufficient money to finance additional work along these lines as project IR-WIO. With assistance from students of the University of New Brunswick Forestry School and Mr. J. H. Hallett of the

Forest Biology Laboratory, Fredericton, one of us (J.C.) was able to extend analyses for water content to other softwoods available at Fredericton. Thus, it was possible to cut and analyze 160 trees of balsam fir; 160 of red spruce, *Picea rubens* Sarg.; 16 of white spruce; and 72 of eastern white cedar, *Thuja occidentalis* L. A preliminary report of this work was made to the P.P.R.I.C. in December, 1953, but a more detailed treatment here forms Sections 3.3, 3.4, and 3.5.

Materials and Methods

The materials used and the methods followed have been relatively uniform and may be summarized here. It will be necessary, however, to add special notes in some cases when discussing individual species. The trees used have been the native species, self-sown and growing under substantially natural conditions in Morgan's Woods (now the Morgan Arboretum of McGill University) in the case of eastern hemlock, and in the University Forest at the University of New Brunswick in the case of yellow birch, balsam fir, red spruce, white spruce, and eastern white cedar. We have concentrated our attention upon all parts of the wood—"butt", "middle", "top", heartwood, and sapwood, and we have also used "bark" (all tissues outside the cambium) from yellow birch and eastern hemlock.

The methods of determining water content have been discussed in previous papers (16, 7, 9) but may be redescribed briefly here. The trees are cut as near the ground as possible and disks are then cut from near the base ("butt"), in the crown of the tree ("top"), and from midway between these ("middle"). The bark is removed and the disks are labelled and weighed immediately to the nearest gram. This gives the "fresh-weight". The disks are then dried in an air-oven at 100°–105° C. and weighed again after they are cooled in a large desiccator. This gives the "oven-dry weight". Water content is then calculated on a dry-weight basis. When separate determinations of the water content of sapwood and heartwood are desired, a second disk is cut in each case. Two segments are marked out on this and these are quickly split out and separated into sapwood and heartwood portions (the sapwood–heartwood boundary is usually clearly marked in freshly-cut samples). These portions are immediately placed in weighed rubber-stoppered vials and weighed on the spot or more commonly in the laboratory. The vials with their contents are then heated like the disk in an air-oven, cooled, and weighed. In some cases (as in yellow birch and eastern hemlock) analyses have proceeded further, sapwood and heartwood being separated into outer and inner zones. Experiments have shown that results from segments give a close approximation to those from whole disks. By adding fresh weights of the segments and adding dry weights of the segments, a calculation of the water content of the whole sample may be made and compared with that from a disk. That is the significance of the columns "disk" and "disk (calc.)" in Tables X–XIII. There may, however, be small but significant differences between samples from the north, east, south, and west sides of the tree, and where very accurate determinations are desired four segments should, perhaps, be taken (p. 232).

When one wishes to calculate the percentages of wood, water, and gas in the tree it is necessary to determine the differences in volume between the wet and dry wood. This is done in a volumeter and this simple instrument and the calculations involved have been described in previous papers (16, 7) (see also p. 223 of the present paper).

At the request of the P.P.R.I.C., we have included in Figs. 7 and 12 to 19 inclusive three lines, X, Y, and Z, to show the buoyancy of wood in water. The lines indicate respectively the water contents at which these woods will (1) just float, (2) float with 25% of their volumes above water, and (3) float with 50% of their volumes above the water surface. These and any other flotation levels can be readily calculated if the shrinkage and specific gravity values are known. An example may be given using the figures for balsam fir in Table 1A of "Canadian Woods" (6).

Shrinkage = 10.8% of green volume
$\therefore$ 1 cc. of green wood = 0.892 cc. when oven-dry
Specific gravity (O.D.) = 0.37
$\therefore$ 1 cc. green wood when oven-dry = 0.330 g.
But 1 cc. of green wood will *just float* when its weight is 1 g.

Hence the difference between 1 g. and the weight of wood substance must be due to water in the wood. This amount is calculated as a percentage of the dry weight thus:

$$X = \frac{1 - 0.33}{0.33} \times 100 = 202\% \text{ moisture content (O.D. wt. basis)}$$

$$Y = \frac{0.75 - 0.33}{0.33} \times 100 = 127\% \quad " \quad " \quad "$$

$$Z = \frac{0.50 - 0.33}{0.33} \times 100 = 52\% \quad " \quad " \quad "$$

Results and Discussion

3.1 Yellow Birch (Betula lutea Michx.)

Method

Unfortunately most, if not all, yellow birch in New Brunswick had been exposed to the dieback conditions by 1949 and it was uncertain whether completely healthy trees were available for study. For this work, therefore, every effort was made to take only those trees that appeared healthy, that is, those that fell into Classes 1A, 1B, and 2A in the crown injury classification originated by Morris (25) and adopted by Hawboldt and Skolko (22).

With the exception of the April 11, 1950, felling, which was from a hardwood stand on Beaver Mt., McAdam, N.B., all trees were cut in a mixed stand in the University of New Brunswick Forest near Fredericton. This area, having uneven topography, represented various exposure and drainage conditions. The trees were selected in early July, 1949, while in full foliage; this aided the choice of healthy specimens. Sizes ranged from 5 to 14 in. D.B.H., with 30% in the 5.0–6.9 in. class, 50% in the 7.0–9.9 in. class, and 20% in the 10.0–14.0 in. class.

The moisture content of trees was determined monthly throughout the year using the technique of Gibbs (7) modified slightly so as to obtain 'weighted' samples (Figs. 1–4). The north side of the standing tree was marked so that it could be used later for the orientation of disk segments. Two disks, 1.25 in. thick, were taken from each of the butt, middle, and top levels; one of each pair was debarked immediately and weighed. These three disks together represent a weighted sample from a (uniformly) tapering trunk, and from them an approximate weighted mean moisture content for the tree trunk can be derived. The second disk from each level was marked off into north, east, south, and west equiangular segments. Each segment was subdivided tangentially into the following functional zones (where present): bark, outer sapwood, middle sapwood, inner sapwood, outer heartwood, and inner heartwood. The entire segment was used up in the zonal samples, whose sum represented a weighted sample of a disk quadrant, the four segments from one disk a weighted sample of one level, and all segments together a weighted sample of a tree trunk. Each zonal sample was immediately enclosed in an air-tight weighed vial (Fig. 4) and its fresh weight obtained. Similarly, samples of the bark and wood of twigs from the upper and lower crown were enclosed in vials and weighed. Dry weights were obtained as previously described, and the moisture content determined on a % dry weight basis. Ninety-one trees were analyzed in this way.

Intermediate fellings for entire disks and twig samples were made during bud-opening and leaf-fall when the moisture content was changing quickly, and during or after exceptionally dry weather. One hundred trees were analyzed thus.

If the density and swelling of wood are known, it is possible to calculate the space occupied by wood substance, water, and gas in the specimens when cut. This often gives a better appreciation of conditions within the living tree than do mere values of water content based on dry weight. For the calculation of the density and swelling of yellow birch wood, the fresh and dry volumes of 54 blocks from 27 trees were measured with a volumeter (Fig. 5). For the conversion of moisture content based on dry weight to approximate moisture content based on fresh volume, a conversion factor is derived from these volumetric data as follows:

$$\text{Per cent moisture (dry wt. basis)} \times \text{conversion factor} = \text{per cent moisture, (wet vol. basis)}$$

$$\text{i.e., } \frac{(\text{wet wt.} - \text{dry wt.}) \times 100}{\text{dry wt.}} \times \text{conversion factor} = \frac{(\text{wet wt.} - \text{dry wt.}) \times 100}{\text{wet vol.}},$$

$$\text{or, conversion factor} = \frac{(\text{wet wt.} - \text{dry wt.}) \times 100}{\text{wet vol.}} \times \frac{\text{dry wt.}}{(\text{wet wt.} - \text{dry wt.}) \times 100},$$

$$= \frac{\text{dry wt.}}{\text{wet vol.}}; \qquad (1)$$

$$\text{but, density oven-dry} = \frac{\text{dry wt.}}{\text{dry vol.}},$$

$$\therefore \text{dry wt.} = \text{density oven-dry} \times \text{dry vol.};$$

$$\text{also, swelling} = \frac{\text{wet vol.}}{\text{dry vol.}},$$

$$\therefore \text{wet vol.} = \text{swelling} \times \text{dry vol.}$$

Substituting these terms in equation (1),

$$\text{conversion factor} = \frac{\text{density oven-dry} \times \text{dry vol.}}{\text{swelling} \times \text{dry vol.}} = \frac{\text{density oven-dry}}{\text{swelling}}. \quad (2)$$

Substituting in equation (2) the figures for mean density and mean per cent swollen volume from determinations on 54 blocks of wood,

$$\text{conversion factor} = \frac{0.644}{1.238} = 0.520.$$

Using Stamm's (31) figure of 1.53 as the density of wood material itself, an example of the application of the conversion factor may be given. Suppose a sample of wood has 68% moisture on a dry-weight basis, then the percentage volumes occupied by wood substance, water, and gas in the green state were:

$$\text{wood substance:} \quad \frac{\text{dry wt.} \times 100}{\text{wet vol.} \times 1.53} = \frac{\text{conversion}}{\text{factor}} \times \frac{100}{1.53} = 34.0\%.$$

$$\text{water:} \quad 68\% \text{ dry wt. basis} \times \text{conversion factor} = 35.4\%,$$

$$\text{gas:} \quad 100 - (34.0 + 35.4) = 30.6\%.$$

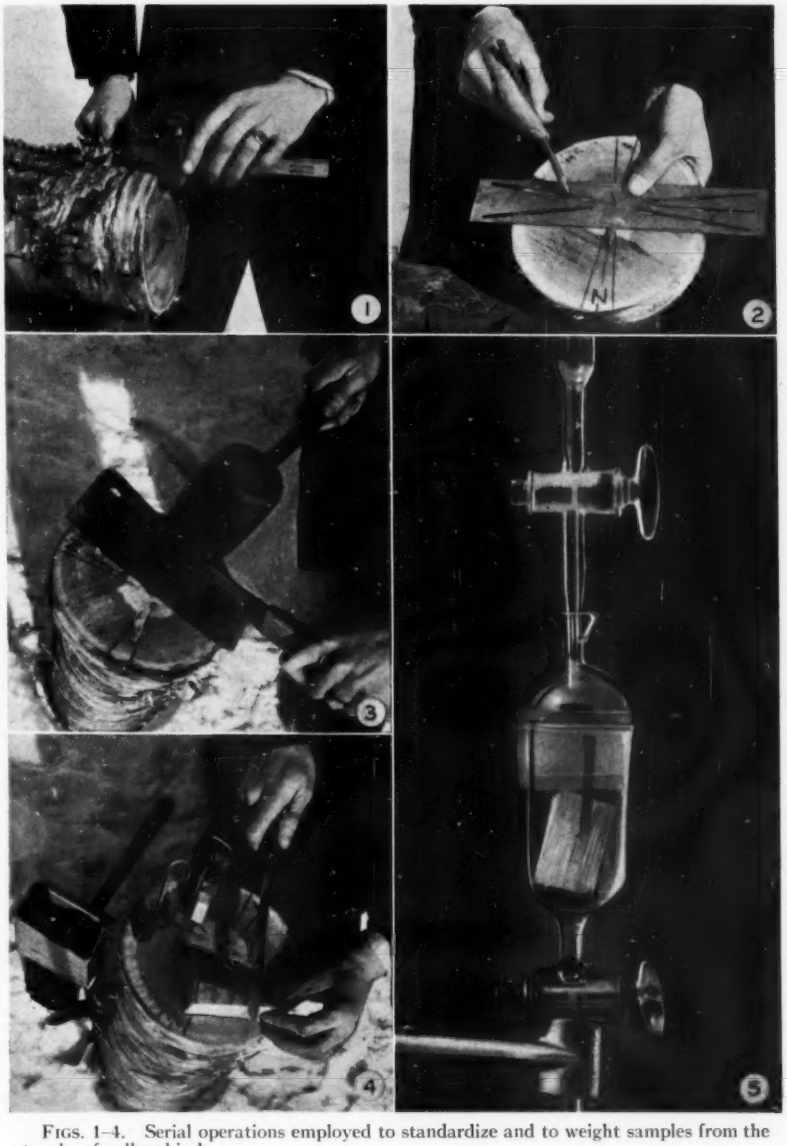
In Table I this is applied to a few of the actual samples to show how well the calculated values (using the conversion factor) agree with the determined values (using the original data—wet weight, dry weight, density, and swelling—measured from the actual samples).

TABLE I

"DETERMINED" AND "CALCULATED" VALUES (FRACTIONS OMITTED) OF PERCENTAGE COMPOSITION BY VOLUME OF GREEN WOOD OF YELLOW BIRCH

Position of sample		Water (% dry wt. basis)	Percentage composition determined			Percentage composition calculated		
Level	Zone		Wood	Water	Gas	Wood	Water	Gas
Butt	O.S.[a]	65	32	33	25	34	34	32
Middle	O.S.	87	34	46	20	34	45	21
Butt	O.S.	81	35	44	21	34	42	24
Butt	O.S.	81	35	44	21	34	42	24
Butt	O.S.	85	34	45	21	34	44	22
Butt	O.H.	83	35	45	20	34	43	23
Butt	I.H.	83	33	43	24	34	43	23
Butt	I.H.	79	33	41	26	34	41	25
Middle	O.S.	84	33	43	24	34	44	22
Middle	O.S.	100	34	50	16	34	52	14
Butt	I.H.	59	34	29	37	34	31	35
Middle	M.S.	78	34	41	25	34	41	25
Top	I.S.	88	35	46	19	34	46	20
Mean			33.8	42.2	24.0	34.0	42.2	23.8

[a]Outer sapwood, outer heartwood, inner heartwood, middle sapwood, and inner sapwood, respectively.



FIGS. 1–4. Serial operations employed to standardize and to weight samples from the trunks of yellow birch.

FIG. 5. Volumeter for measuring volumes of fresh and dry wood samples.

The conversion is based on the assumption that density and swelling are constant. This, of course, is not true, but the error involved is not great in yellow birch and does not detract from the usefulness of the factor as a means of conveniently arriving at approximate volumetric relationships.

To eliminate the effects of variables such as site and exposure that are unavoidable in analyses of felled trees and which might be confused with the influence of weather on moisture content, twig samples from a group of eight trees were analyzed twice monthly (except in the winter months) from July 1949 to November 1952.

McDermott noted (24), and Gibbs confirmed (14) his observation, that the method of cutting twigs has a measurable effect on their water content. This effect, which could not be conveniently eliminated in this work, was standardized by adhering to the following cutting procedure. A twig about 18 in. long was removed from the end of a mid-crown branch by a pole pruner. The distal and then the proximal portions of the twig were clipped off leaving a sample about 6 in. long and 0.25 in. in diameter. This was divided into two or three short lengths which were quickly debarked; their wood was then enclosed in one air-tight vial, their bark in another. Fresh weights were determined in the laboratory. As far as possible all samples were collected between 11.00 a.m. and 2.30 p.m.

Total Water Content of the Trunk

A knowledge of the total water content of living trees has applications in logging and in the conversion of logs to various forest products. Standardization of the disk thickness gives samples weighted only by their radii. It is therefore possible to estimate the total water content of the tree from the sum of the green weights and the sum of the dry weights of the disks from butt, middle, and top. Obviously, this figure is influenced to a great degree by the volume of the butt-log. The solid line in Fig. 6A records the seasonal total water content of the wood of the trunk derived in this way.

If the segments give a true picture of the water content at a given level, they may be used instead of disks to provide a similar picture for the entire trunk, provided that the segment angle is standardized—as it was in this section of our work (Fig. 2). The broken line in Fig. 6A is derived from segments and it is clear that there is close agreement with the results derived from whole disks.

In late April almost half the weight of the trunk is due to water (90% water on a dry-weight basis = 47% on a fresh-weight basis), while in September water accounts for but one-third of the weight (50% water on a dry-weight basis = $33\frac{1}{3}$% water on fresh-weight basis). These results, when expressed in terms of percentage composition, yield the curves given in Fig. 6B. At the time of maximum moisture content (late April) almost one-half of the volume of the trunk is water: five months later (September) only one-quarter of the volume is water. In late June and again soon after leaf-fall, the volumes of wood, water, and gas are nearly equal.

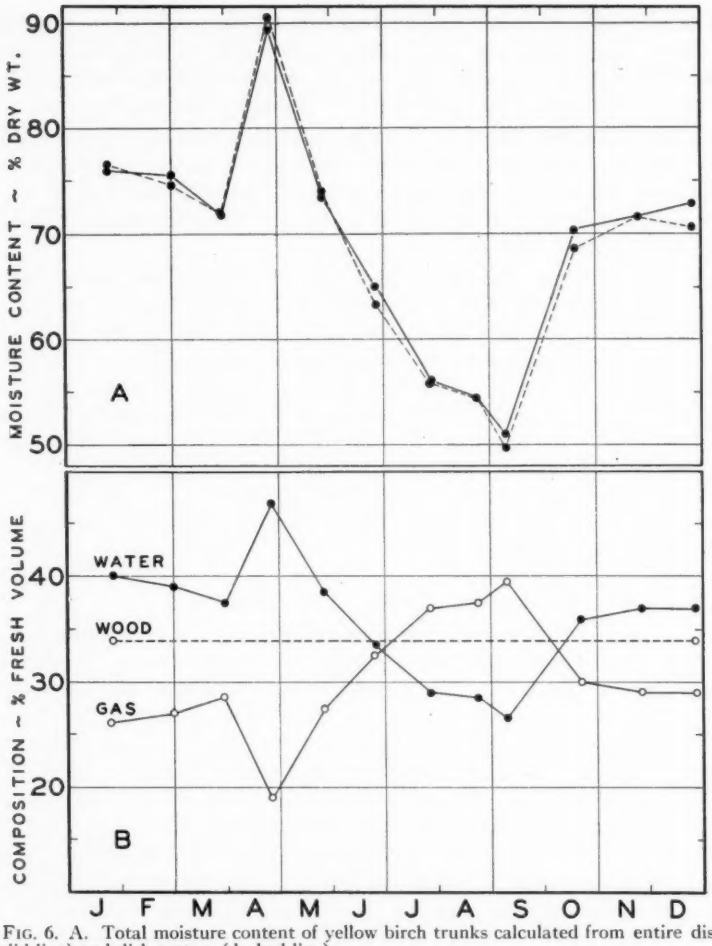


FIG. 6. A. Total moisture content of yellow birch trunks calculated from entire disks (solid line) and disk sectors (dashed line).
B. Seasonal relationship of wood, water, and gas contents in trunks of yellow birch.

The flotation of yellow birch wood in water (Fig. 7), though of less practical interest to the woods operator in this mechanized age than formerly, exhibits interesting features which might apply to other common hardwood species. Unseasoned wood of twigs which will just float in late February, March, and September will sink when cut at any other time of year. During April and May unseasoned logs from all heights in the trunk will immediately sink. The 50% flotation line *Z* (see p. 222 for explanation) does not appear in Fig. 7 because it is impossible for a high specific gravity wood like yellow birch to float at this level. Theoretically, the maximum flotation for yellow birch wood (oven-dry) would be with about 44% of its volume above water.

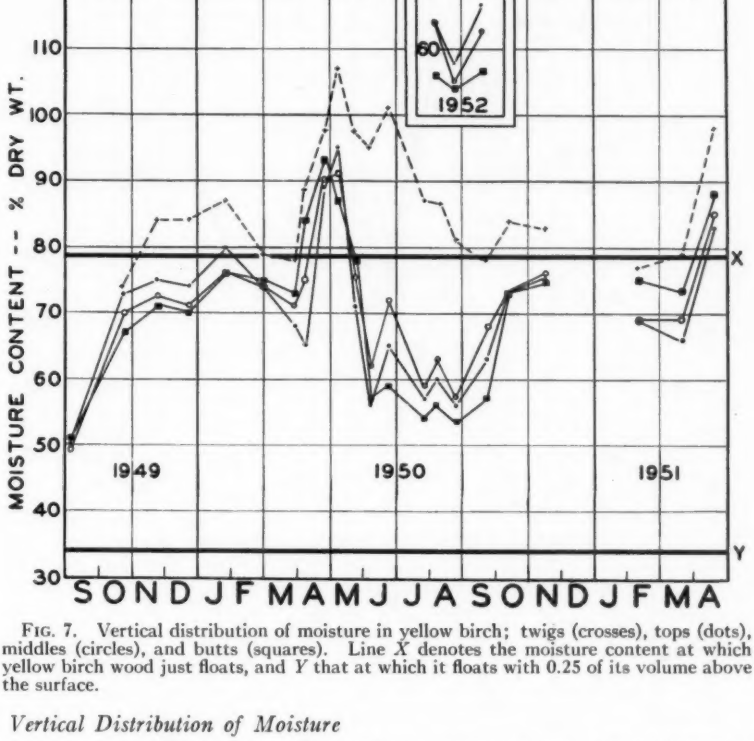


FIG. 7. Vertical distribution of moisture in yellow birch; twigs (crosses), tops (dots), middles (circles), and butts (squares). Line *X* denotes the moisture content at which yellow birch wood just floats, and *Y* that at which it floats with 0.25 of its volume above the surface.

Vertical Distribution of Moisture

Of less practical value than the total water content but of more interest to the physiologist are the seasonal patterns of vertical and lateral distribution of moisture within the tree. From the vertical water content data (Table II, and Fig. 7) it is noted that two seasons particularly stand out as possible stress periods. One of these occurs at the end of the winter and is most pronounced high up in the trunk; the other occurs in late summer after a gradual loss of water from the entire tree during the growing season. For comparable dates in different years, the available data conform to a more or less regular seasonal pattern. For example, the patterns of water content for March and April of 1950 and 1951 are almost identical; also the late-summer minima for 1950 and 1952 are very similar. There is a marked similarity between these results and those of Gibbs (9, 12) for white birch and wire birch. This supports Gibbs' (15) observation that though genera may differ considerably from each other, species within a genus closely follow the same pattern of seasonal water content. For ease of comparison Gibbs' results for wire and white birch are shown in Fig. 8 along with the yellow birch averages from Table II.

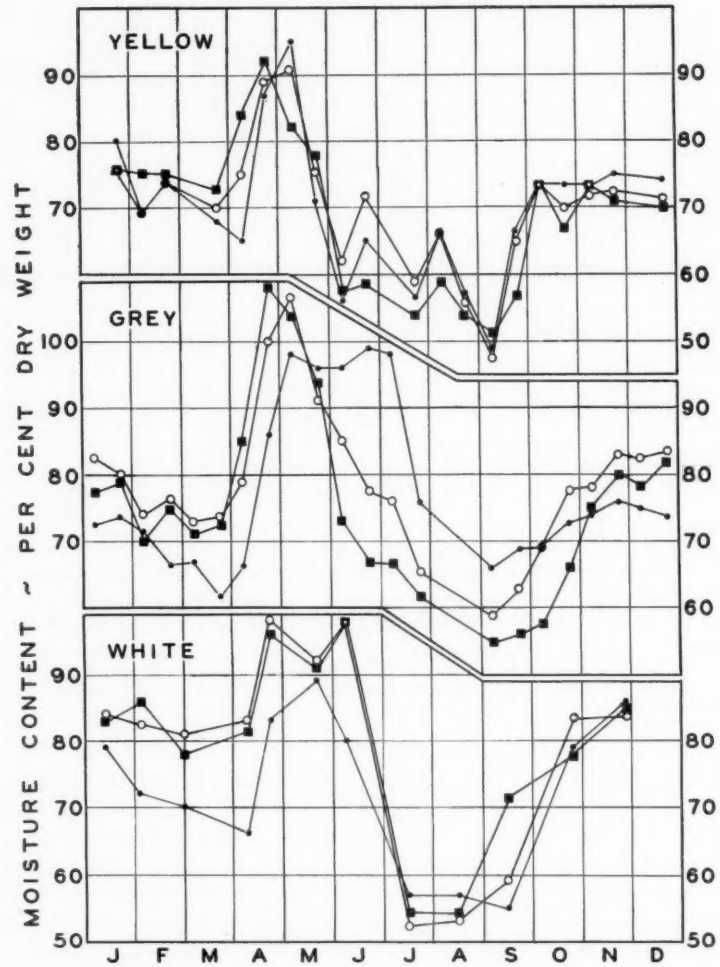


FIG. 8. Seasonal pattern of moisture in the trunks of yellow, gray, and white birch; tops (dots), middles (circles), and butts (squares). (Curves for white and gray birch from published data, Gibbs 1935 and 1939.)

If the average moisture data in Table II are converted from a dry-weight basis to a fresh-volume basis we find that during bud-swelling and bud-breaking, the water content rises from 34% in early April to 49% by early May, then by early June falls to 29% of the total volume of the upper trunk while over the same two-month period the volume occupied by gas diminishes from 32% to 17%, then increases to 37%. In early May, water accounts for over one-half (55.6%) of the volume of twig wood.

TABLE II

MEAN WATER CONTENT OF WOOD DISKS AT THREE LEVELS IN YELLOW BIRCH TREES CUT NEAR FREDERICTON, 1949–1952

Dates	1949				1950				1951				1952				Averages			
	B	M	T	N	B	M	T	N	B	M	T	N	B	M	T	N	B	M	T	N
Jan. 15–30	—	—	—	—	76	76	80	8	—	—	—	—	—	—	—	—	76	76	80	8
Feb. 1–15	—	—	—	—	—	—	—	—	75	69	69	4	—	—	—	—	75	69	69	4
Feb. 15–28	—	—	—	—	75	74	74	8	—	—	—	—	—	—	—	—	75	74	74	8
Mar. 15–31	—	—	—	—	73	71	68	9	73	69	69	5	—	—	—	—	73	70	68	14
Apr. 1–15	—	—	—	—	84	75	65	8	—	—	—	—	—	—	—	—	84	75	65	8
Apr. 15–30	—	—	—	—	93	90	89	10	88	85	83	4	—	—	—	—	92	89	87	14
May 1–15	—	—	—	—	87	91	95	7	—	—	—	—	—	—	—	—	87	91	95	7
May 15–31	—	—	—	—	78	76	71	10	—	—	—	—	—	—	—	—	78	76	71	10
June 1–15	—	—	—	—	57	62	56	6	—	—	—	—	—	—	—	—	57	62	56	6
June 15–30	—	—	—	—	59	72	65	11	—	—	—	—	—	—	—	—	59	72	65	11
July 15–31	—	—	—	—	54	59	57	10	—	—	—	—	—	—	—	—	54	59	57	10
Aug. 1–15	—	—	—	—	63	60	60	6	—	—	—	—	56	64	64	10	59	62	62	16
Aug. 15–31	—	—	—	—	54	57	56	10	—	—	—	—	54	55	58	10	54	56	57	20
Sept. 1–15	51	48	49	2	—	—	—	—	—	—	—	—	—	—	—	—	51	48	49	2
Sept. 15–30	—	—	—	—	57	68	63	6	—	—	—	—	57	63	67	10	57	65	65	16
Oct. 1–15	—	—	—	—	73	73	73	4	—	—	—	—	—	—	—	—	73	73	73	4
Oct. 15–31	67	70	73	8	—	—	—	—	—	—	—	—	—	—	—	—	67	70	73	8
Nov. 1–15	—	—	—	—	75	76	72	4	71	68	74	5	—	—	—	—	73	72	73	9
Nov. 15–30	71	72	75	8	—	—	—	—	—	—	—	—	—	—	—	—	71	72	75	8
Dec. 15–31	70	71	74	8	—	—	—	—	—	—	—	—	—	—	—	—	70	71	74	8

NOTE: B = Butt. M = middle. T = top. N = number of trees.

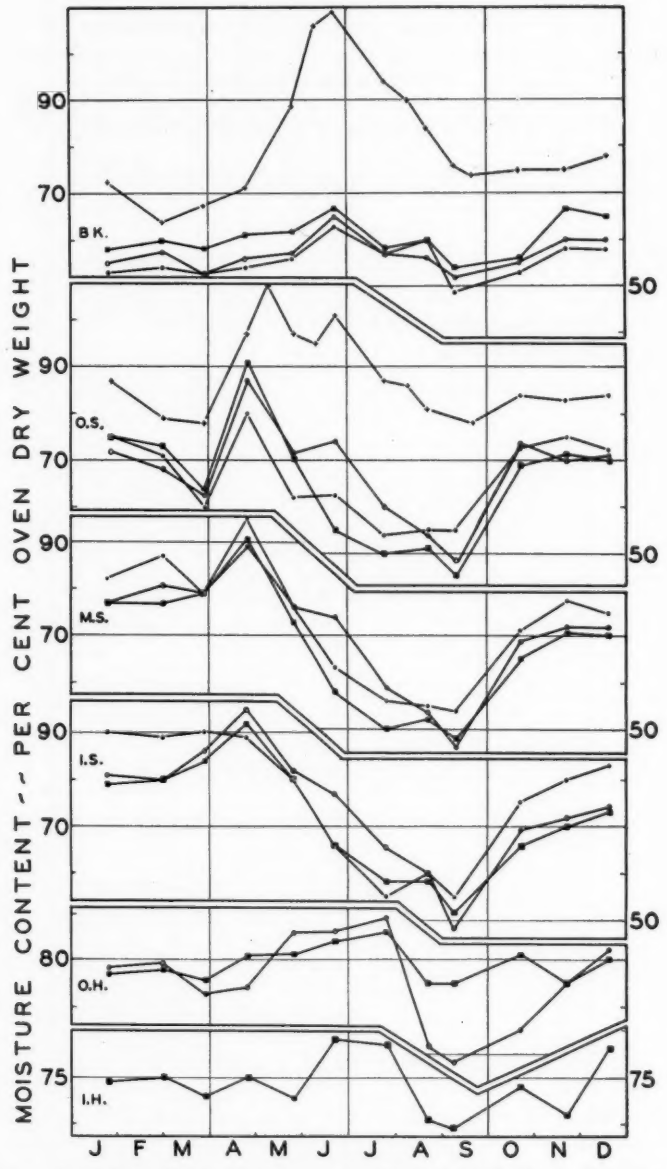


FIG. 9. Lateral distribution of moisture in yellow birch twigs (crosses), tops (dots), middles (circles), and butts (squares): B.K. = bark; O.S. = outer, M.S. = middle, and I.S. = inner sapwoods, respectively; O.H. = outer and I.H. = inner heartwoods.

Lateral Distribution of Moisture

The lateral distribution of moisture in the trunks (Fig. 9) is determined from the small samples into which each disk segment was split.

Water content of the trunk-bark changes only slightly while that of the twig-bark has extreme seasonal variations. The difference is no doubt largely due to differences in the relative amounts of functional and total bark present. Total bark here implies everything exterior to the xylem but functional bark includes only the active phloem and cambial tissues. This is supported by the analysis of bark water content (Table III) which shows that the difference between levels is mostly due to differences between twig- and trunk-bark. Similarly, significant interaction of levels and dates is entirely due to variability of seasonal changes in twig versus trunk bark, rather than at different levels within the trunk. The maximum water content of the bark occurs 6 to 7 weeks after the maximum for wood, that is, the bark is wettest at the time when most of the new leaves are fully expanded and functionally active. This seems to be true of the barks of nearly all the species examined by Gibbs (15, Fig. 5).

Of all wood zones the outer sapwood shows the greatest seasonal fluctuations of moisture content. Here, too, the late-winter minimum is most pronounced. The March minima of moisture content of twig and top outer sapwood are respectively as low as and only 5% higher than the September minima, whereas the middle has 17% and the butt has 18% more water in the outer sapwood in March than in September. In the middle sapwood zone the winter minimum is less pronounced but the summer minimum is almost as low as that of the outer sapwood. On the other hand, in the inner sapwood

TABLE III

ANALYSIS OF VARIANCE OF MOISTURE CONTENT OF BARK FROM TWIGS AND TRUNKS OF YELLOW BIRCH

Source	D.F.		M.S.
Between trees			
Dates	10		911**
Error (*a*)			
(Trees same date)	77		80
Within trees			
Levels	3		10526**
Trunk levels		2	550
Trunk vs. twig levels		1	30476**
Interactions			
Levels × dates	30		280**
Trunk levels × dates		20	20
Twig vs. trunk levels × dates		10	800**
Error (*b*)			
(Trees same date × levels)	231		26
Total	351		

**Difference significant at 1% level.

zone the late-winter minimum disappears entirely but the summer minimum is not greatly different from those above. Thus the low water content in late winter is most pronounced in the outer wood zones high up in the tree. These observations point to wind and low atmospheric humidity as important factors in the winter loss of moisture. By contrast, the summer minima are more or less the same throughout the three sapwood zones and appear to be governed by some internal factor, such as total water uptake, or breaking of water columns, influencing the conduction of water. Greenidge (17) has shown that in healthy yellow birch the entire cross section of sapwood appears capable of functioning in water conduction. Evidence that solar radiation is still another factor in the winter loss of water is presented below. The curves for sapwood, uninfluenced by heartwood values, clearly show that a steady decrease of water in the conducting tissue occurs through the growing season and results in a possible stress period before the natural shedding of leaves.

The heartwood itself has no great extremes of moisture content, although both the outer and inner zones rise in mid-summer to subdued maxima which are probably the result of lateral diffusion of water from the wet sapwood.

Effect of Solar Radiation on Moisture Content

The variation of moisture content from one segment to another within a disk follows a seasonal pattern in which the south side of the trunk plays a prominent part. Data for bark and sapwood of winter, spring, and summer fellings are summarized in Table IV to show the deviation of the south sector from the mean of the four cardinal directions. In January, all zones are drier on the south side. The differences diminish generally from outer to inner

TABLE IV

DEVIATIONS OF THE SOUTH MOISTURE CONTENT FROM THE MEAN MOISTURE CONTENT OF NORTH, EAST, SOUTH, AND WEST QUADRANTS FOR BARK AND THREE SAPWOOD ZONES OF YELLOW BIRCH

		Mean deviation of moisture content (% dry wt.) of south from mean of north, east, south, and west			
Date 1950	Sample	Bark	Outer sapwood	Middle sapwood	Inner sapwood
January 24 (8 trees)	Top	−2.1	−6.3	−2.0	−1.8
	Middle	+0.3	−9.0	−1.1	−1.0
	Butt	−1.8	−3.4	−0.3	−0.5
	Tree	−1.2	−6.2	−1.1	−1.0
April 24 (8 trees)	Top	−1.5	−7.5	−1.4	+0.9
	Middle	−0.4	−6.0	+1.8	0.0
	Butt	−1.8	−4.3	−0.5	+2.0
	Tree	−1.2	−5.8	0.0	+1.0
July 24 (8 trees)	Top	+2.1	+0.6	−0.3	−0.8
	Middle	+0.4	0.0	+1.6	+1.0
	Butt	−0.8	+1.1	+0.9	+0.1
	Tree	+0.6	+0.6	+0.7	+0.1

sapwood and mostly from top to butt. Late in April, when the trunk as a whole is wettest (Fig. 6A), the bark and outer sapwood of the south side are still drier at all levels than those of the other three sectors, but the drying effect does not extend into the middle and inner sapwood zones. By July the differences are inconsistent and small. The external factor which best explains the results is solar radiation. Through winter and spring the trunk is exposed to direct sunshine but in summer it is shielded by the foliar canopy.

From Table IV it appears that the directional effect is most pronounced in the outer sapwood zone. An analysis of variance of all outer sapwood data for eight fellings (January to August, inclusive) is presented in Table V. The significant difference between quadrants is shown by the refined analysis to be almost entirely due to highly significant differences between the south and the other three quadrants. The highly significant interaction, dates × quadrants, indicates that the differences between quadrants averaged over all levels are not uniform over all dates. In other words the directional pattern of moisture distribution changes with the season. The analysis supports the explanation of moisture loss from the south side of trunks during the leafless condition of the trees.

Moisture Content of Twigs

The mean moisture content of twigs from eight trees (Fig. 10) follows a seasonal pattern similar to that of entire trees. In twigs the winter and summer minima are about equal, both being approximately 70% (dry weight basis). The maximum water content of twig bark occurs several weeks after that of twig wood. In general, the bark contains less moisture than the wood during the leafless condition of the tree, and more than the wood during the foliage season.

TABLE V

ANALYSIS OF VARIANCE OF MOISTURE CONTENT OF OUTER SAPWOOD FROM FOUR DIRECTIONS AT EACH OF THREE LEVELS

Eight monthly fellings, January–August, 1950, inclusive

Source	D.F.	S.S.		M.S.	F
Dates	7	9,525.63		1,360.80	$\frac{1.360.80}{109.67^a} = 12.4^{**}$
Levels	2	211.22		105.61	1.20
Quadrants	3	325.36		108.45	4.47*
S. vs. rest		1	287.40	287.40	11.85**
W. vs. N. + E.		1	32.49	32.49	1.34
N. vs. E.		1	5.47	5.47	<1
Dates × levels	14	1,233.35		88.10	32.75**
Dates × quadrants	21	509.54		24.26	9.02**
Levels × quadrants	6	49.75		8.29	3.08
$D \times L \times Q$	42	113.11		2.69	
Total	95	11,967.96			

[a]From components of variance thus: $\sigma^2 + 3\sigma_{DQ}^2 + 4\sigma_{DL}^2$,
$= 2.69 + 21.57 + 85.41,$
$= 109.67.$

*Difference significant at 5% level.
**Difference significant at 1% level.

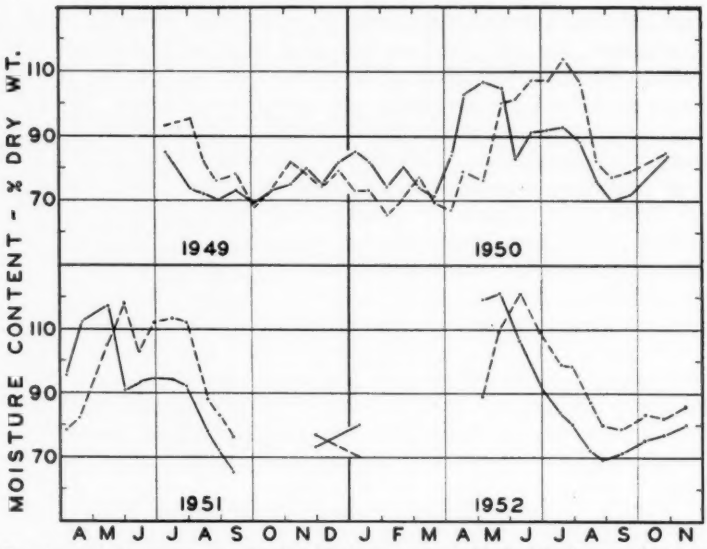


FIG. 10. Moisture content of yellow birch twigs: bark (dashed line), wood (solid line).

TABLE VI

AVERAGE MOISTURE CONTENT (% DRY WT.) OF THE WOOD AND BARK OF YELLOW BIRCH TWIGS FROM EARLY JUNE TO EARLY SEPTEMBER, 1950, 1951, AND 1952

	Year	June 6 to June 13	June 28 to July 6	July 16 to July 18	July 30 to Aug. 7	Aug. 16 to Aug. 21	Sept. 6 to Sept. 15	Average***
		Average of eight trees*						
Wood	1950	82.8	92.0	92.6	90.0	76.1	69.6	83.9
	1951	92.8	94.0	92.8	92.4	78.0	64.8	85.9
	1952	106.0	92.3	83.6	79.8	72.1	70.8	84.1
	Av.**	93.8	92.8	90.0	87.4	75.4	68.4	
Bark	1950	101.1	106.9	113.6	111.4	82.1	77.0	98.7
	1951	103.1	111.7	113.5	114.1	88.2	75.9	101.1
	1952	121.0	109.3	98.8	98.5	86.6	78.8	98.8
	Av.**	108.4	109.3	108.6	108.0	85.7	77.2	

No. measurements in an average		Significant differences between averages			
		Wood		Bark	
		P .05	P .01	P .05	P .01
*	8	10.0	13.2	11.5	15.2
**	24	5.7	7.6	6.6	8.8
***	48	4.1	5.4	4.7	6.2

NOTE: Solid brackets indicate highly significant differences between comparable fellings in different years. Dotted brackets indicate significant differences between comparable fellings in different years.

It is of interest to compare the observed maximum moisture content of twig wood (121% on a dry-weight basis in May, 1952) with the theoretically highest possible value for yellow birch wood of average density (127%) calculated by means of the conversion factor 0.52 (p. 223). Obviously the wood approached and perhaps reached saturation at this time.

Superimposed on the seasonal rise and fall of moisture content (Fig. 10) are minor variations of the pattern which suggest the influence of some local factor. For example, curves for 1950 and 1951 are very similar but differ considerably from those of 1952 and the incomplete data of 1949. The averages of moisture content of twigs are given in Table VI and differences between means that are statistically significant at the 1% and 5% levels are also shown. Though the yearly averages are very similar, there are certain periods when the average for 1952 is significantly different from averages of 1951 and 1950. It is notable that the twigs were decidedly drier in late July and early August of 1952 despite the fact that they were also significantly wetter in early June of that year.

Meteorological records for the summers of 1949–1952 inclusive (Table VII) show below average precipitation for 1949 but above average rainfall in the other 3 years. However, temperatures were above average in both 1949 and 1952; thus it is probable that the evaporating power of the air in those years was also above average. We are indebted to Dr. C. W. Thornthwaite for calculating the water balance (Fig. 11) according to a new concept (33) which is a modification of his earlier method of climatic analysis (32). Both 1949 and 1952 were years of high potential evapotranspiration and small water deficits occurred in spite of the above normal summer rainfall in 1952. In the years between, the summer precipitation plus soil moisture just balanced the summer water need. The trees probably did not suffer by the water shortages of 1949 and 1952, for greater deficits have occurred in the past, for example in 1946, 1939, 1921, and 1903, with no apparent ill effects. It is of interest to note, however, that the mild climatic stress periods of 1949 and 1952 seem to be reflected in the moisture content of twigs.

The Water Economy and Dieback of Yellow Birch

This study, though carried on in a period which did not include a good 'test' year of drought, indicates two periods when dry conditions are likely to have maximum effect on the water economy of yellow birch. One of these

TABLE VII

SUMMER RAINFALL AND MEAN TEMPERATURE AT FREDERICTON, 1949 TO 1952, DEVIATIONS FROM AVERAGE (FROM MONTHLY WEATHER RECORD, DEPARTMENT OF TRANSPORT, CANADA)

	Average		Deviations from average							
			Rainfall, in.				Temperature, °F.			
	Rainfall, in.	Temp., °F.	1949	1950	1951	1952	1949	1950	1951	1952
June	3.41	60.7	−1.06	+2.10	+0.11	+2.54	+2.2	−0.9	−1.4	+1.4
July	3.02	66.8	−0.91	+0.38	+3.54	−0.97	+1.1	−2.4	−0.2	+4.1
August	3.61	64.2	−0.99	−0.33	+0.77	−0.80	+1.8	−0.8	+0.6	+2.4
Summer			−2.96	+2.15	+4.42	+0.77	+5.1	−4.1	−1.0	+7.9

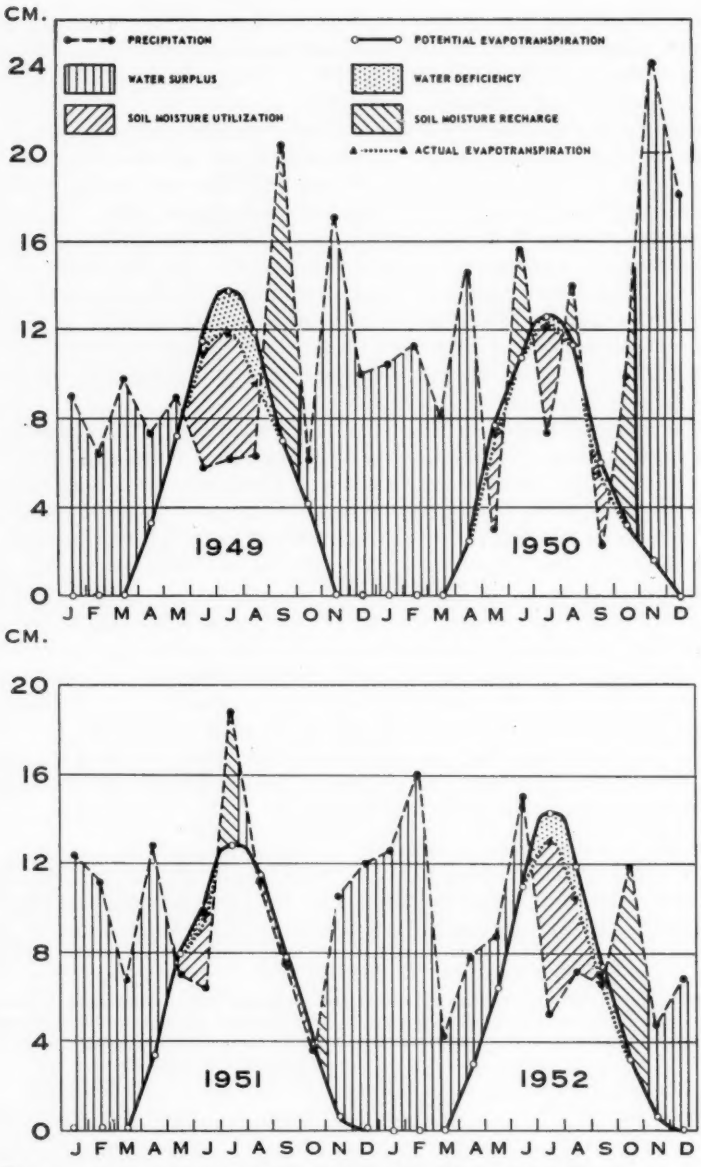


FIG. 11. The water balance at Fredericton, N.B., 1949 to 1952 inclusive.

'stress' periods occurs in late winter when the water content of the tree has been reduced by evaporation. This drying out is most pronounced in the twigs and outer sapwood of the upper trunk. The other 'stress' period appears in late summer when (if the tension hypothesis is invoked) many of the water columns have broken and the vessels which held them are gas-filled. This depletion of the moisture content occurs throughout the sapwood and, to some extent, in the outer heartwood. Late summer thus seems to be the more hazardous of the two stress periods. This knowledge is of importance in any study of, or interpretation of, the role of drought in the initiation of dieback. Water deficiencies are not simply the result of low precipitation but are also related to excessive evapotranspiration induced by high temperature. When water deficiencies develop in Eastern Canada they occur in July, August, or September, and thus coincide with a potential stress period in the moisture regime of yellow birch. Two seasons during the course of the study, 1949 and 1952, did have water deficits which seemed to disturb slightly the moisture regime of twigs but there is no evidence that these or other water-deficient summers since 1897 might have been drastic enough to initiate the dieback of birch. These findings point out, however, that times of high water need should be taken into consideration in any analysis of meteorological data designed to discover drought periods that may have been responsible for the deterioration of a certain species.

After completion of the field work, circumstances delayed publication of our results. In the meantime, accounts of other investigations of birch dieback by Greenidge (17), Redmond (27, 28), and others appeared in the literature. Greenidge showed that the advanced stages of dieback are accompanied by a disturbed moisture distribution in the stem. He postulated that high rootlet mortality reduces water uptake, resulting in the death of twigs and branches, which in turn reduces the transpirational surface in the crown. He suggests that the combined effects of these factors result in subnormal moisture in the top and above normal moisture in the butt.

Redmond reports that changes in temperature upset the balance of fungal populations in the root and rhizosphere and disturb the normal development of mycorrhiza. He showed that small sustained artificial increases in average soil temperature (2° C. for 100 days) caused about 50% mortality of yellow birch rootlets and suggests that the trend of higher summer temperatures in Eastern Canada could conceivably have raised the average soil temperature as much as 2° C.

If increased temperature should be responsible for initiation of birch dieback, the temperature factor probably plays a much more complex role in the physiology of yellow birch than has been hitherto suggested. However, in any one area the over-all changes in climate tend to be very small during the life of a birch tree when compared to either the macro- or micro-climatic variations from place to place within the natural range of the species. Nevertheless, birch dieback has occurred on hilltops and in valleys, on north slopes and on south slopes. It has for some time now failed to advance farther

TABLE VIII

Tsuga canadensis—MORGAN'S WOODS, 1936–1943

AVERAGE WATER CONTENT (% DRY WT.) OF GROUPS OF 10 TREES

Av. date	Av. D.B.H., in.	Bark			Wood														
					Disk			Butt				Middle				Top			
		B	M	T	B	M	T	Out. spw.	In. spw.	Out. htw.	In. htw.	Out. spw.	In. spw.	Out. htw.	In. htw.	Out. spw.	In. spw.	Out. htw.	In. htw.
Jan. 19	6.0	63	87	116 (4)	96	109	119	118	89	84	100	137	104	94	96	144	108	96	87
Feb. 20	6.1	71	91	107 (5)	98	110	121	116	97	90	100	133	110	96	97	141	114	105	102
Mar. 16	6.1	61	89	98 (5)	96	106	116	114	86	86	100	132	105	81	96	134	105	95	99
Apr. 13	6.1	62	93	109 (6)	90	102	119	94	76	92	98	115	89	98	113	136	105	111	115
May 15	6.1	65	90	103 (6)	90	94	103	92	74	87	99	114	81	85	90	121	85	100	99
June 14	6.2	62	93	106 (5)	96	109	119	121	80	89	95	135	102	100	99	144	98	104	103
July 13	5.9	65	96	114 (8)	95	113	121	113	76	86	101	138	100	107	108	153	99	105	101
Aug. 22	6.0	61	95	116 (8)	94	103	115	110	72	94	101	122	91	10[illegible]	98	135	101	113	97
Sept. 19	5.9	66	94	113 (6)	87	103	115	97	71	82	104	117	90	10[illegible]	101	137	100	108	101
Oct. 17	6.0	66	97	114 (6)	93	108	120	103	80	93	107	126	101	98	111	136	107	110	110
Nov. 18	6.2	65	96	122 (4)	101	113	133	121	86	96	99	138	109	97	110	158	118	114	107
Dec. 18	6.1	68	101	117 (4)	104	115	129	127	91	88	104	144	106	96	89	155	116	105	97

NOTE: Figures in parentheses are numbers of trees whose bark was analyzed.

westward into the warm continental climate of the Great Lakes Region but is still active (although much less so than previously) in the cooler maritime climate of northern Cape Breton Island. All of this re-emphasizes the need for a more realistic physiological approach to the study of climate in forest problems.

3.2 Eastern Hemlock (Tsuga canadensis (L.) Carr.)

This conifer, which reaches its northern limits about 100 miles north of Montreal, grows to a fairly large size at the western end of the Island of Montreal in a region studied by Long (23). He concluded that the vegetation of the area is borderline between the Great Lakes – St. Lawrence mixed forest of Halliday (18) and the deciduous beech–maple forest of Braun (5).

Between November 21, 1936 (Expt. S.A. 36.35) and March 13, 1943 (Expt. S.A. 43.3) a total of 127 trees, ranging from 5 to 8.5 in. D.B.H. and averaging about 30 ft. in height, were cut as occasion offered. They were sampled by the disk method at butt, middle, and top; two segments were also taken in each case from extra disks and divided into outer and inner sapwood and outer and inner heartwood; in addition, the water content of bark was determined for 76 of the trees. The results of these studies are summarized in Tables VIII and IX and Figs. 12, 13, and 14. It should be noted that for the statistical analysis seven trees are omitted and 12 groups of 10 are dealt with.

TABLE IX

ANALYSES OF VARIANCE OF MOISTURE CONTENT OF OUTER AND INNER ZONES OF SAPWOOD AND HEARTWOOD OF HEMLOCK

Source	D.F.	Sapwood, M.S.	Heartwood, M.S.
Bet. dates	11	4520**	1140
Bet. levels	2	44943**	4625**
Bet. zones	1	184256**	2880**
$D \times L$	22	250	259
$D \times Z$	11	582	238
$L \times Z$	2	986	3622**
$D \times L \times Z$	22	332	447
Replicates + error	648	362	654
Total	719		

**Difference significant at 1% level.

The water content of hemlock varies greatly from tree to tree as is well brought out in Fig. 3 of Gibbs (15), where results from individual trees, as well as averages, are plotted. There are indications, however, of a seasonal pattern with minima in May and August–September and of a vertical gradient with highest average values in tops and lowest average values in butts. When results from the different zones, outer and inner sapwood and outer and inner heartwood, are considered (Fig. 13) it becomes even clearer that there is a distinct seasonal pattern in the sapwood, but that this is not reflected in the heartwood. Vertical and lateral gradients are also indicated. This is confirmed by statistical analyses (Table IX) which indicate highly significant

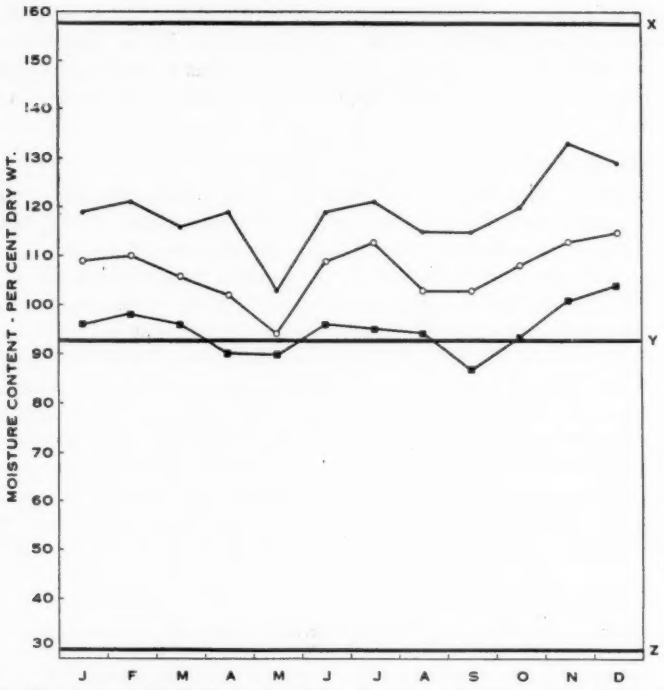


FIG. 12. Vertical distribution of moisture in eastern hemlock: tops (dots), middles (circles), and butts (squares). $X = 0\%$, $Y = 25\%$, $Z = 50\%$ flotation; for explanation see p. 222.

differences between dates, due to the seasonal pattern; between levels, due to the vertical gradient; and between zones, due to the horizontal distribution of moisture content. The heartwood shows highly significant differences between levels but not between dates—there is, as inspection of Fig. 13 shows, no real seasonal pattern in the heartwood. The inner heartwood is significantly wetter than the outer heartwood.

A point worth noting is that hemlock resembles balsam fir (Gibbs (9) and p. 246 of this paper) in having wet and dry patches, rather than zones, in the heartwood. In balsam the wet patches may be completely full of water: the driest patches may have little more water than is required to saturate the cell walls. No attempt was made to separate wet and dry patches in the case of hemlock but some of the samples were largely one or the other. The wettest heartwood sample taken had 180% water: the maximum possible water content of hemlock is just under 200%. We have recorded 214% in sapwood (of presumably less than average density). The driest heartwood sample had 43% water, which is not very different from the constant values for the consistently "dry" heartwoods of jack pine and white spruce as recorded by Gibbs (9).

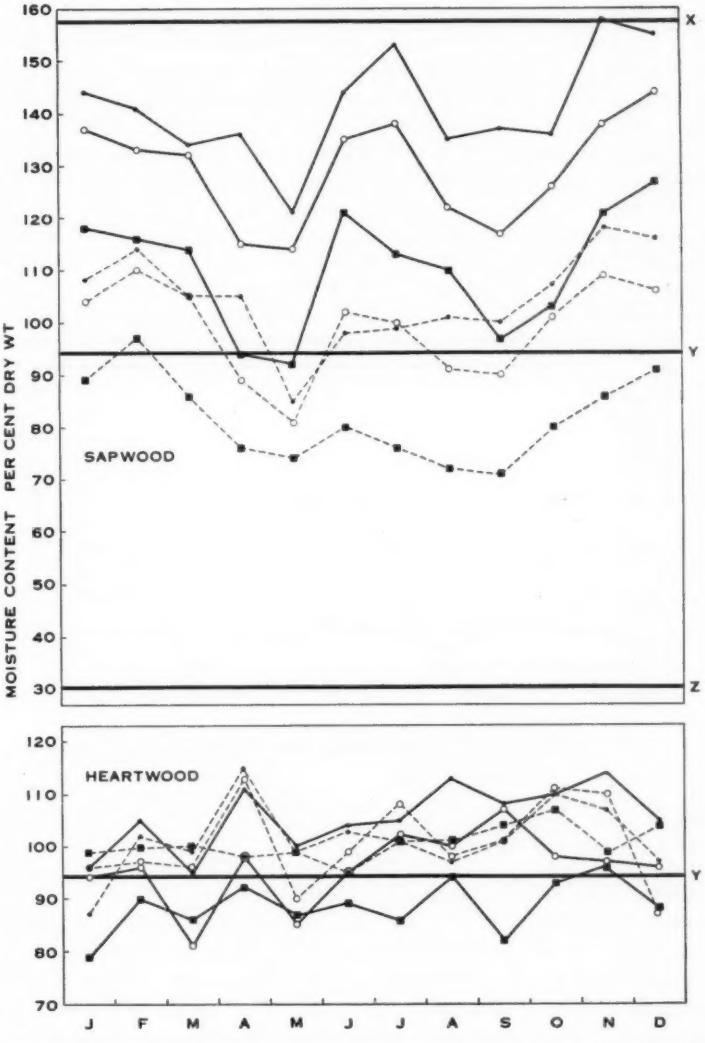


FIG. 13. Lateral distribution of moisture in eastern hemlock: tops (dots), middles (circles), butts (squares); outer zones (solid lines), inner zones (dashed lines). $X = 0\%$, $Y = 25\%$, $Z = 50\%$ flotation; for explanation see p. 222.

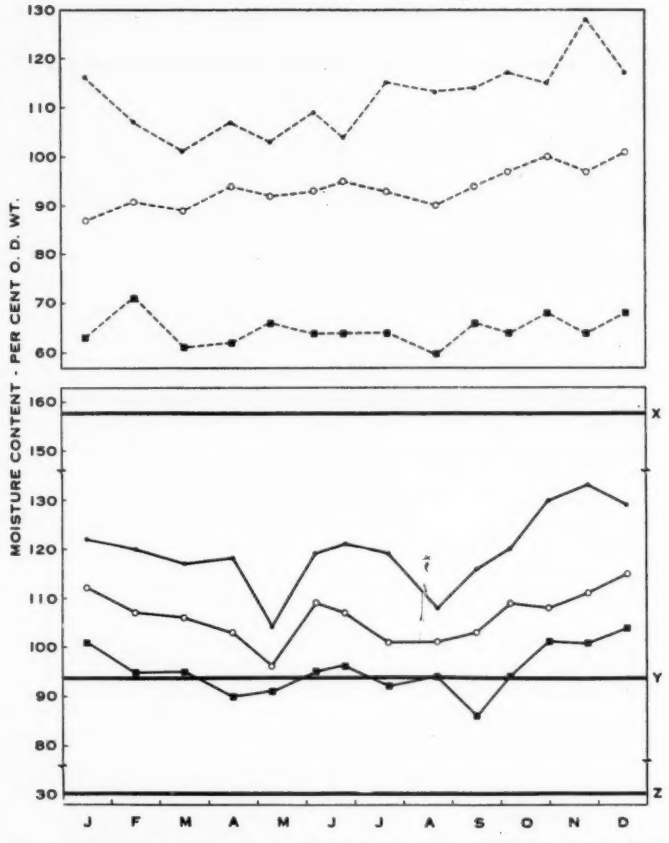


FIG. 14. Moisture content of bark (dotted) and wood (solid) of butts (squares), middles (circles), and tops (dots) of eastern hemlock. X = 0%, Y = 25%, Z = 50% flotation; for explanation see p. 222.

3.3 Balsam Fir (Abies balsamea (L.) Mill.)

Between December 1, 1951, and November 9, 1952, a total of 160 trees, ranging from 5 to 12 in. D.B.H. and averaging 45 ft. in height, were cut in a fir–spruce stand in the University of New Brunswick Forest. Accurate identification was not possible in all cases (owing to the absence of female cones) but it is likely that many of these trees were bracted balsam fir (var. *phanerolepis* Fern.), which is common in the Fredericton district.

Care was taken to avoid trees which were obviously damaged by the balsam woolly aphid, *Adelges piceae* (Ratz.), which is prevalent in central New Brunswick. However, it was frequently found after felling a tree that the upper portion of the stem bore evidence of a light infestation of the insect.

These trees were discarded only if the middle and top disks contained much "rotholz", a peculiar type of wood formation (1) which results from stem attacks.

Cuts were made, as indicated in Table X, and disks were taken as usual from butt, middle, and top in each case, the average water content being recorded under "disk". Segments were cut from additional disks at butt and middle, for the determination of sapwood and heartwood water contents. Since these segments were taken as described on p. 221 it was also possible by summing fresh weights and summing dry weights in each case to calculate what the water content of a disk "should" have been. This provided a check on the figures from complete disks. The average results are recorded under the heading "disk (calc.)" in Table X. It will be noted that the "disk" and "disk (calc.)" figures usually agree very well. The results are summarized in Figs. 15 and 16.

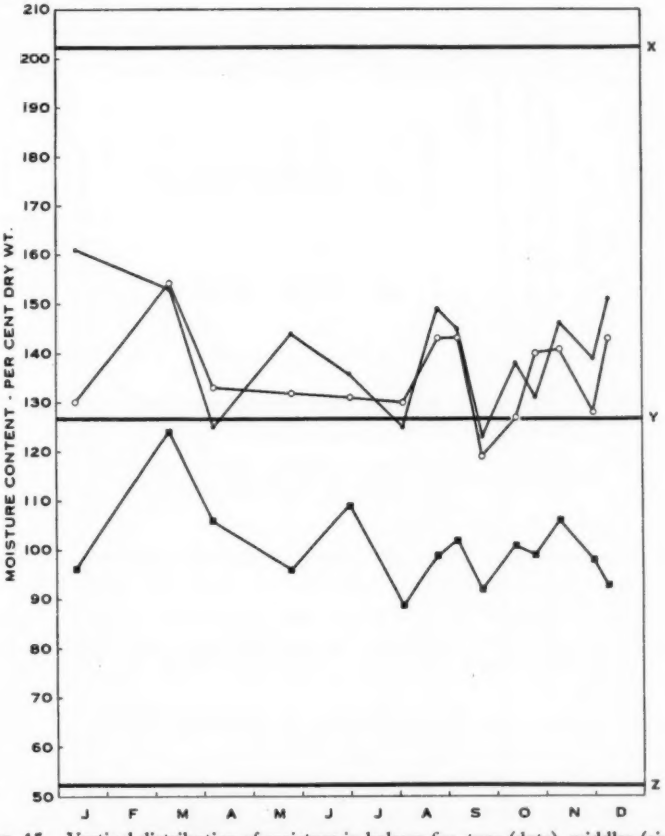


FIG. 15. Vertical distribution of moisture in balsam fir; tops (dots), middles (circles), and butts (squares). $X = 0\%$, $Y = 25\%$, $Z = 50\%$ flotation; for explanation see p. 222.

TABLE X

AVERAGE WATER CONTENT (% DRY WT.) OF WOOD OF BALSAM FIR, FREDERICTON, N.B., 1951–52.
TWELVE TREES PER FELLING UNLESS OTHERWISE STATED

	D.B.H., in.	Butt				Middle				Top	Remarks
		Disk	Disk (calc.)	Spw.	Htw.	Disk	Disk (calc.)	Spw.	Htw.	Disk	
1951											
Dec. 1	7.5	98	102	186	66	128	132	187	96	139	
Dec. 8	7.3	93	97	166	59	143	144	194	109	151	
1952											
Jan. 12	7.2	96	94	178	54	130 132* (10)	135 137* (10)	187	95	161	11 trees
Mar. 8	7.8	124	121	184	73	146 154* (11)	139 155* (9)	167 189* (11)	114	147 153* (11)	
Apr. 5	7.7	106	112	165	75	133	144 137* (11)	167 147* (11)	122	125	
May 24	6.5	96	98	138	73	132	136	157	118	144	8 trees
July 1	6.8	99	98	151	68	131	122	159	96	136	8 trees
Aug. 2	6.8	89	91	157	57	130	125	153	106	125	
Aug. 23	6.5	99	98	153	58	143	141	183	112	149	
Sept. 6	7.0	102	103	153	72	143	150	186	122	145	
Sept. 21	6.9	92	94	153	69	119	123 121* (11)	147	106	123	
Oct. 12	7.5	101	109	184	71	127	130	167	110	138	
Oct. 25	7.7	99	105	171	66	140	141	179	112	131	
Nov. 9	6.8	106	106	160	74	141	137	178	108	146	

*Average with doubtful results omitted. Parentheses show numbers of items in starred averages.

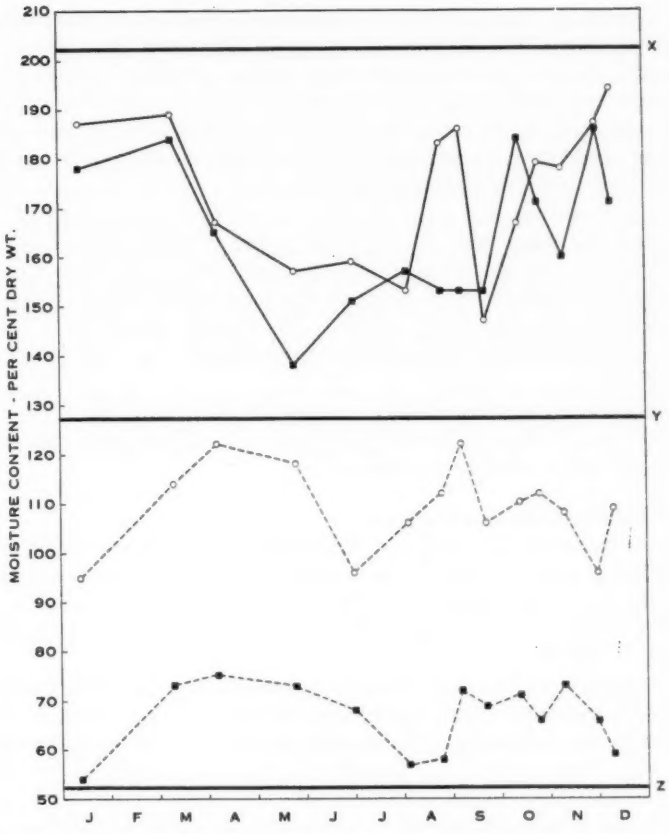


FIG. 16. Lateral distribution of moisture in the boles of balsam fir trees: middles (circles), butts (squares), sapwood (solid lines), and heartwood (dashed lines). $X = 0\%$, $Y = 25\%$, $Z = 50\%$ flotation; for explanation see p. 222.

It must be remembered that many of these results were obtained by groups of relatively inexperienced forestry students and a few were obviously wrong since they indicated impossibly high water content or did not agree where they should (the "disk" and "disk (calc.)" figures). Omitting these questionable results we have recalculated average figures and indicated these by stars in Table X.

The analysis of variance of moisture content (including all results) (Table XIV) shows that there are highly significant differences between dates and between levels owing to the seasonal and vertical distribution of water in the trees. The highly significant interaction indicates that the levels do not behave alike on different dates.

The occurrence of wet and dry patches in the heartwood of balsam fir, noted and discussed by Gibbs (9), was evident again in the trees from Fredericton, but no effort was made to separate them in our analyses. It would be of considerable interest to survey balsam from all over its area of distribution to see whether there are varieties (strains, forms) which lack the wet patches. Such trees would have a heartwood of relatively low water content—probably less than 40%—and would be good floaters.

The maximum possible water content for wood of average density was calculated to be about 260% by Gibbs (9, p. 742). Calculations from Table 1A in "Canadian Woods" (6) suggest a figure of 236%. There are figures for sapwood in our Table X which approach the lower of these figures, suggesting almost completely filled wood. Gibbs (9) recorded figures over 300%—presumably in specimens of less than average density, i.e., with more room for water.

The sapwood of the trees cut above Chicoutimi was definitely much wetter than that of the Fredericton trees as reported here. This is rather surprising, and it is difficult to offer an explanation.

3.4 Red Spruce (Picea rubens Sarg.)

Between November 27, 1951, and November 10, 1952, a total of 160 trees, ranging from 5 to 11 in. D.B.H. and averaging 46 ft. in height, were cut in a red spruce – fir – cedar stand on a flat, moist site in the University of New Brunswick Forest. The results of these fellings are summarized in Table XI and Figs. 17 and 18.

Fig. 16 shows that tops were consistently much wetter than the middles and that these in turn were a little wetter than the butts. This is due, of course, to the decreasing proportion of sapwood and increasing proportion of heart-

TABLE XI

AVERAGE WATER CONTENT (% DRY WT.) OF WOOD OF RED SPRUCE, FREDERICTON, N.B., 1951–52. TWELVE TREES PER FELLING EXCEPT WHERE INDICATED

Date	D.B.H., in.	Butt				Middle				Top
		Disk	Disk (calc.)	Spw.	Htw.	Disk	Disk (calc.)	Spw.	Htw.	Disk
1951										
Nov. 29	8.2	99	94	137	41	110	112	159	54	144
Dec. 8	7.0	79	79	123	41	93	98	142	42	127
1952										
Jan. 12	6.3	96	95	142	37	110	112	160	37	146
Mar. 8	7.8	83	81	128	42	95	96	150	46	130
Apr. 5	7.3	82	86	125	39	91	96	140	42	125
May 24	6.3*	83	87	127	36	91	92	127	40	127
July 1	6.5*	65	66	104	40	72	72	108	38	115
Aug. 3	6.4	63	63	93	35	75	73	114	36	112
Aug. 24	7.0	72	76	123	39	88	88	136	37	141
Sept. 7	6.9	79	86	120	33	97	100	136	35	126
Sept. 20	7.8	78	83	128	34	86	88	132	33	130
Oct. 5	—	67	69	107	35	75	78	118	36	131
Oct. 26	7.3	66	71	108	36	76	82	130	39	134
Nov. 10	7.5	78	81	126	40	93	96	137	40	146

*Eight trees.

wood found in a top to butt examination. In red spruce, as in the white spruce (*Picea glauca* (Moench) Voss) examined above Chicoutimi by Gibbs (9), where it was labelled by the synonym *P. canadensis*, the heartwood is consistently dry. That of white spruce had a water content of about 40%; that of the red spruce reported here had a little less. There is no doubt that some of the "heartwood" listed in Table XI included a little sapwood: it is not always easy in the field—and particularly under winter conditions—to make an accurate separation.

There is also in Fig. 16 a suggestion of a seasonal fluctuation in water content, with minima in June–July and again in October. This invites comparison with hemlock (Figs. 12 and 13) where minima appear in May and August–September. Analysis of variance (Table XIV) shows that these differences are highly significant. That the seasonal fluctuations are due to changes in water content of the sapwood rather than the heartwood is made clear by Fig. 18 where heartwoods of butt and middle logs (tops had little or no heartwood) are shown to be consistently "dry".

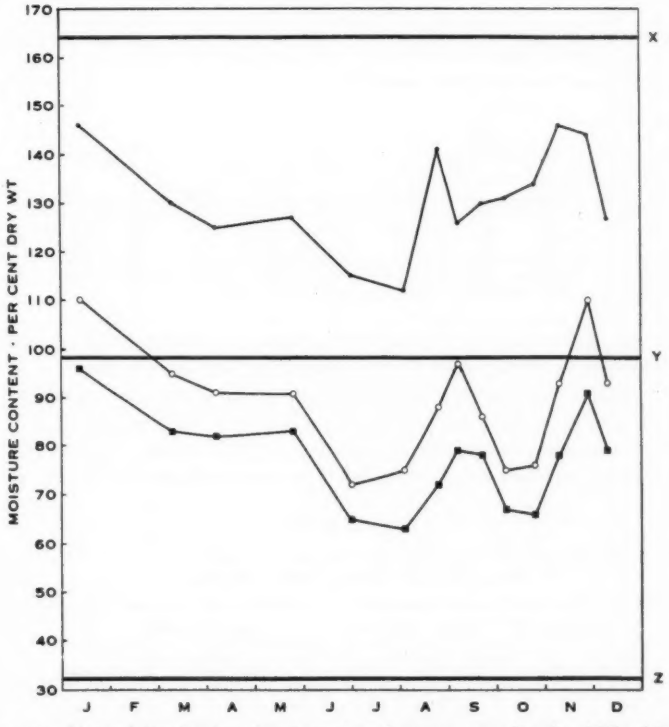


FIG. 17. Vertical distribution of moisture in red spruce: tops (dots), middles (circles), and butts (squares). $X = 0\%$, $Y = 25\%$, $Z = 50\%$ flotation; for explanation see p. 222.

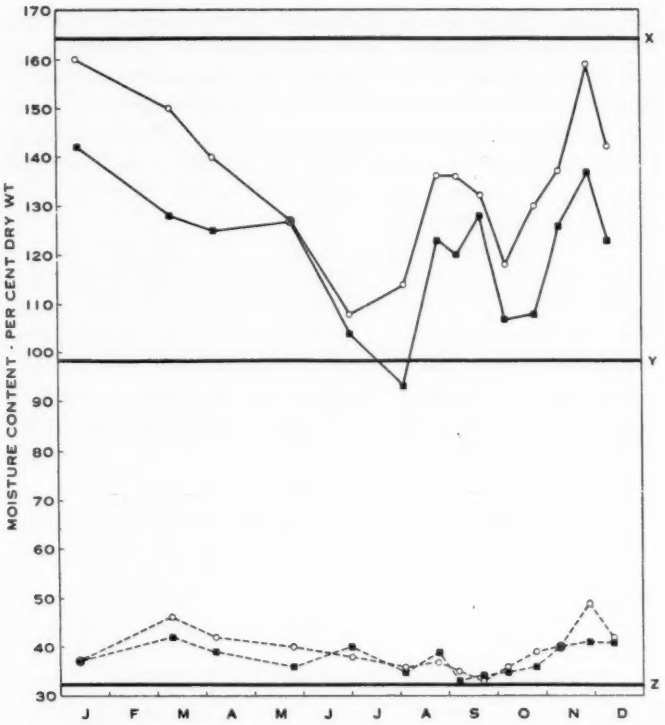


FIG. 18. Lateral distribution of moisture in the boles of red spruce trees: middles (circles), butts (squares), sapwood (solid lines), and heartwood (dashed lines). $X = 0\%$, $Y = 25\%$, $Z = 50\%$ flotation; for explanation see p. 222.

From the figures given in Table 1A of "Canadian Woods" (6) a maximum water content of 198% may be calculated for red spruce wood of average density. Water content in sapwood indicating complete saturation was recorded only in one or two samples in the present investigation. White spruce, on the other hand, can "hold" about 220% water. Gibbs (9) found values well below this in the trees which he examined above Chicoutimi.

TABLE XII

AVERAGE WATER CONTENT (% DRY WT.) OF WOOD OF WHITE SPRUCE, FREDERICTON, N.B., 1951–52. EIGHT TREES PER FELLING

Date	D.B.H., in.	Butt				Middle				Top
		Disk	Disk (calc.)	Spw.	Htw.	Disk	Disk (calc.)	Spw.	Htw.	Disk
1951										
May 31	6.4	88	99	136	47	104	108	150	44	163
July 12	6.9	102	106	162	48	121	128	160	46	152

3.5 White Spruce (Picea glauca (Moench) Voss)

Only two fellings (each of eight trees) of this species were made, on May 31 and on July 12, 1952. The results are summarized in Table XII. They are too few to be of much use but it is noteworthy that the July 12 figures are higher than those of May. The reverse result was obtained for red spruce. Both sapwood and heartwood figures compare closely with those noted by Gibbs (9) for white spruce trees cut north of Chicoutimi.

3.6 Eastern White Cedar (Thuja occidentalis L.)

Between June 14 and November 11, 1952, a total of 72 trees ranging from 5 to 12 in. D.B.H. and averaging 35 ft. in height were cut in the stand in which red spruce was also being sampled for water content (Section 3.4). They were treated as were the balsam fir, red spruce, and white spruce described above. The results are summarized in Table XIII and Fig. 19.

There are indications of minima in July and September corresponding with those noted for red spruce. That the seasonal differences noted are statistically significant is clearly brought out in Table XIV. There are also

TABLE XIII

AVERAGE WATER CONTENT (% DRY WT.) OF WOOD OF EASTERN WHITE CEDAR, FREDERICTON, N.B., 1951–52. EIGHT TREES PER FELLING

Date	D.B.H., in.	Butt				Middle				Top
		Disk	Disk (calc.)	Spw.	Htw.	Disk	Disk (calc.)	Spw.	Htw.	Disk
June 14	6.9	99	95	248	33	103	105	245	30	165
July 20	7.9	83	83	257	37	89	92	238	33	150
Aug. 9	7.5	100	101	290	38	102	106	243	33	170
Aug. 23	6.6	90	93	257	31	109	112	240	29	174
Sept. 7	7.6	81	82	245	37	93	95	219	30	162
Sept. 21	7.4	82	83	229	34	80	84	184	28	147
Oct. 5	8.5	90	93	262	36	91	95	235	29	154
Oct. 19	7.4	91	92	236	34	94	100	215	28	172
Nov. 11	6.9	90	87	244	34	109	111	241	31	152

TABLE XIV

ANALYSES OF VARIANCE OF MOISTURE CONTENT OF WOOD DISKS FROM TOPS, MIDDLES, AND BUTTS DURING THE FOLLOWING PERIODS: BALSAM FIR AND RED SPRUCE, DEC 1951–NOV. 1952; HEMLOCK, NOV. 1936–MARCH 1943; CEDAR, JUNE 1952–NOV. 1952

	Balsam fir		Red spruce		Eastern hemlock		Eastern white cedar	
	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Between trees								
Dates	13	1,731**	13	3,477**	13	957	8	1,413**
Error (*a*) (individuals same date)	146	522	146	705	113	706	63	462
Within trees								
Levels	2	73,466**	2	125,694**	2	17,827**	2	110,848**
L × *D*	26	6,281**	26	372**	26	274**	16	302**
Error (*b*) (individuals same date × levels)	292	169	292	88	226	103	126	161
Total	479		479		380		215	

**Difference significant at 1% level.

highly significant differences in water content as between the tops and other parts of the trees, but not between middles and butts which have much the same water contents. This is, of course, due to the great proportion of sapwood in tops as compared with middles and butts. The sapwood in eastern white cedar is very wet indeed. Calculations using the figures in Table 1A of "Canadian Woods" (6) show that the maximum water content for wood of average density is about 268%. We have recorded many figures in excess of this and in August the *average* water content for the sapwood of butt logs (eight trees) was 290%, suggesting that our trees were full of water and had wood of less than average density.

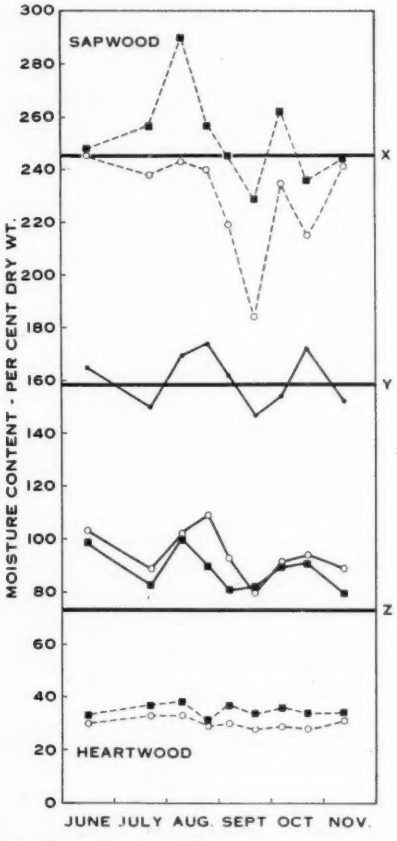


FIG. 19. Summer and fall pattern of moisture in eastern white cedar (*Thuja occidentalis*): tops (dots), middles (circles), butts (squares), whole disks (solid lines), sapwood and heartwood (dashed lines). $X = 0\%$, $Y = 25\%$, $Z = 50\%$ flotation; for explanation see p. 222.

The heartwood of these trees, on the other hand, contains very little water—probably less than 30%—for we frequently logged values of 27–30%. This is a little lower than we have recorded for heartwood of white and red spruce, jack pine, and the driest "dry patches" of the heartwood of balsam fir.

Summary

The seasonal changes in water content of yellow birch are strikingly like those of wire and white birches, which occasions no surprise. There is evidently a great similarity in the physiologies of these related trees. The recorded changes in moisture content examined in the light of climatic data seem to lend little support to the view that dieback is due to an increase in temperature or to drought, except possibly through their effects on some unknown biotic factor. Much work is necessary on the physiology of the tree and the combined effects of precipitation, temperature, humidity, and insolation before definite conclusions can be drawn. Thornthwaite's method of analyzing climatic data and the measurement of evapotranspiration are important contributions in this direction. The periods of low water content in the spring and fall suggest times of stress at which reduced availability of water and increased demand for water would be most serious. It is to be hoped also that the question of so-called minor element deficiencies will receive further attention in the near future.

The seasonal changes in water content of the conifers studied here are evidently relatively slight, as might be expected from their evergreen habit. It is our ambition to investigate *Larix*, *Metasequoia*, and *Taxodium*, which, being deciduous, might well resemble the hardwoods more closely than do the typical softwoods. We have some figures for twigs and for a very few whole trees of *Larix* which resemble those of hardwoods, but more data are needed.

Tsuga canadensis shows a regular seasonal rhythm in the water content of its outer sapwood, with lows in April–May and in August–September. Its heartwood is interestingly like that of *Abies balsamea* in having wet and dry patches. It would be useful to know if there are varieties of these trees which have consistently dry heartwoods; they would be much better floaters. It would be interesting, too, to know if other species of *Tsuga* and *Abies* also have wet patches. One is tempted to predict that they do.

The seasonal changes in water content of the conifers studied here are so slight that little use can be made of them in minimizing loss by sinkage. Owing to high water content of its heartwood *Tsuga canadensis* is a poor floater at any season. *Abies balsamea* and *Picea rubens* depend largely on their heartwoods for flotation, hence large logs with a high heartwood–sapwood ratio are the best floaters. This is particularly true in balsam fir where the heartwood of the butt is not only larger but consistently drier than that of the middle of the tree (Fig. 16). For the best flotation of green balsam fir the trees should be cut in July and August. In red spruce the heartwood is uniformly dry but the sapwood has two periods of low water content, July and October, hence felling of trees should be made at these times for maximum

buoyancy of green logs. Lastly, on the basis of our incomplete seasonal data for *Thuja occidentalis* we might predict all-year-round excellent floating qualities for this species because of its uniformly very dry heartwood, its high heartwood–sapwood ratio, and the low specific gravity, 0.31 (6), of its wood.

Acknowledgments

This work was financed in part by a grant from Science Service, Department of Agriculture, Canada, towards a co-operative research project with McGill University, Montreal, to study the water requirements in relation to 'dieback' of yellow birch. Financial assistance was also given by the Pulp and Paper Research Institute of Canada to extend the work to include studies of the moisture content of coniferous trees.

The authors express sincere thanks to all those who assisted in any way, and particularly to Dr. R. E. Balch, Officer-in-Charge, Forest Biology Laboratory, Fredericton, N.B., and Prof. F. K. Hare, Chairman, Department of Geography, McGill University, for their helpful suggestions and stimulating interest in the work; to Dr. C. W. Thornthwaite, Director, the Laboratory of Climatology, Elmer, New Jersey, for kindly revising calculations of the water balance at Fredericton; and to Dr. J. Miles Gibson, Dean of Forestry, University of New Brunswick, for providing most of the trees required.

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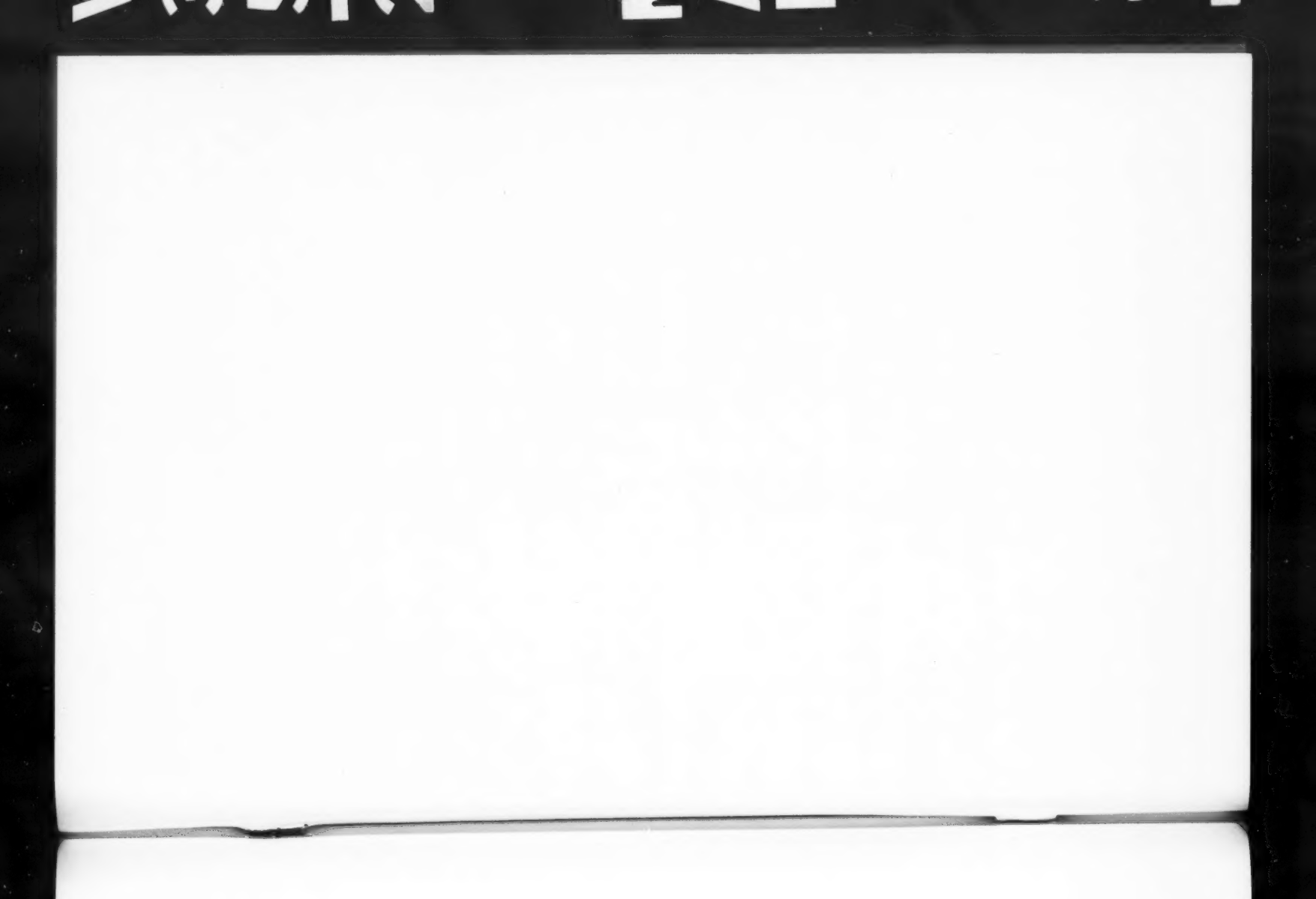

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